Kristu Jayanti Journal of Core and Applied Biology (KJCAB)
Volume 2, Issue 1, 2022, Pages 1-9 | Original Research

Biosorption of Hexavalent Chromium using Fungal Strains Isolated from Soil

Shruthi Cyriac, Needhu John and Elcey C. Daniel*

Department of Life Sciences, Kristu Jayanti College, Autonomous, Bengaluru, Karnataka, India-560077

ABSTRACT

Chromium tolerant fungal strains were isolated from heavy metal contaminated environments and identified using morphological and microscopic observation followed by ribotyping. The microorganisms with the maximum tolerance were analyzed by inoculating each fungus in a PD medium supplemented with hexavalent Chromium salts. The presence of the metal was estimated by the acidic reaction with the 1, 5-diphenylcarbazide method using a spectrophotometer (UV – Vis) at 540 nm. Among all the fungal strains, the most tolerant under maximum enrichment was identified as Paecilomyces lilacinus by a zone of inhibition on the PDA medium. The effect of environmental parameters like pH and temperature on the growth and heavy metal removal also were analyzed. Among the fungal strains, *Aspergillus niger, A. terreus, and Paecilomyces lilacinus* could tolerate Chromium (VI) toxicity up to 800 mgL-1. All the fungi were able to completely remove the heavy metal Cr (VI) at the concentration of 10mg/L, where the maximum growth was obtained. However, the organisms could remove the Chromium in higher concentrations at a slow rate. The maximum removal was recorded at acidic pH (4.0) under ambient temperature (30oC). The fungal species, *Paecilomyces lilacinus* exposed to UV- radiation for up to 10 minutes, enhanced its bio-sorption efficiency compared to the normal strain.

KEYWORDS: Chromium biosorption, *Paecilomyces lilacinus* , Fungal bioremediation

Article History | Received: 12. 08. 2022; Revised: 15. 10. 2022; Accepted: 16. 10. 2022; Published Online: 23. 12. 2022

1. INTRODUCTION

Soil, in both rural and urban environments, is the crucial component, and land management is the key to its quality. Mining, manufacturing, and the use of synthetic products, i.e., pesticides, paints, batteries, industrial waste, and land application of industrial or domestic sludge, etc., can result in heavy metal contamination of urban and agricultural soils. Soil and water pollution is a widely recognized global environmental threat, and remediation is a challenge *(Dhankhar & Hooda 2010).* It is caused by the presence of xenobiotic chemicals or other alterations in the natural soil environment. In recent years, heavy metal pollution has become one of the most serious environmental problems in both developed and developing nations of the world. Anthropogenic activities and industrial operations contributed to the increased levels of heavy metal pollution in the environment *(Oyewole et al., 2019).*

Heavy metal contamination of soil is widespread due to metal processing industries, tannery, combustion of wood, coal, and mineral oil, traffic, and plant protecting agents like pesticides/ insecticides, which contain Arsenic as an active ingredient, and fields that had past applications of wastewater or municipal sludge, areas in or around mining waste piles and tailings, industrial areas where chemicals may have been dumped on the ground, or in areas downwind from industrial sites. The toxic effects of heavy metals result mainly from the interaction of metals with proteins (enzymes) and the inhibition of metabolic processes. In contrast to organic pollutants, metals are not mineralized by microorganisms but can be oxidized or reduced, transformed to different redox stages, and usually form a complex with organic metabolites. The heavy metals that enter the food chain leading to bioaccumulation and may pose a risk to human health. Statescu and Cotiusca-Zauca, 2006 described the urban activities that cause soil degradation and the management practices that protect the functions urban societies demand from the soil *(Statescu & Cotiusca-Zauca, 2006).* They sed on heavy metal components that contaminate soil contamination. Acute poisoning from heavy metals is rare through ingestion or dermal contact. Chronic problems associated with long-term heavy metal exposures, Lead to mental lapse, Cadmium to kidney, liver, GI tract, and Arsenic to skin poisoning, kidneys, central nervous system, etc. The presence of heavy metals in traces is toxic and detrimental to both flora and fauna. They are Lead, Zinc, Copper, Chromium, Mercury, Iron, Manganese, Cadmium, Vanadium, Antimony, Arsenic, and Cobalt.

Efforts to adopt physical methods to decontaminate the metal -polluted soil have been initiated for many years and the various methods employed are extraction using mineral acids *(Bhattacharyya & Cheng, 1987).* This treatment process may lead to the destruction of soil due to acidification and high costs of acids. Alternative remediation strategies to reduce the bioavailability of metals are immobilization with repeated addition of substances such as Carbonate, Phosphate, Apatite, Zeolite, Clay minerals, Peat, or Humus and bioleaching with heterotrophic microorganisms. The quest for the most cost-effective method of removing heavy metals is directed towards bio-sorption, which is a physiochemical process that occurs naturally in certain organisms, which allows it to concentrate passively and bind contaminants onto their cellular structure (*Padmavathy, 2003).* This process can be of high value because it will provide an economical alternative for

*Author for correspondence: Elcey C. Daniel | Email: elcey@kristujayanti.com

removing toxic heavy metals from contaminated soil and aid in environmental remediation *(Alluri et al., 2007).* The living cells are less sensitive to metal ion concentration which can grow without any additional supply of nutrients, and are well adapted to ambient conditions of pH and temperature with a low operating cost and generation of minimum biological sludge. Ayele and others studied the utilization of fungal biomass for the removal of heavy metals from the environment *(Ayele et al., 2021).* Various fungi have been investigated for their biosorption efficiency, *i.e. Mucor rouxii (Yan & Viraraghavan, 2000), Rhizopus arrhizus (Subudhi & Kar, 2008), Polyporus squamosus (Wuyep et al., 2007)*, *Aspergillus niger* (*Tereshina et al., 1999; Rostami & Joodaki, 2003*), *Rhizomucor pulsillus* (*Christov et al., 1999*), *Phanerochaete chrysosporium* (*Sing & Yu, 1998), Aspergillus fumigatus* (*Bhainsa & D'Souza, 1999*), *Ganoderma lucidum (Muraleedharan & Venkobachar, 1990).* The present investigation envisages the isolation and identification of fungal strains with biosorption ability from the vicinity of metal industries and other metal utilization sites.

2. MATERIALS AND METHODS

2.1 Initial Screening

Soil samples were collected from the selected sites where metal utilization activities were intense. The collected soil samples were brought to the laboratory and analyzed for the quantity of Cr (VI) metal by a heavy metal assay using the 1, 5 - Diphenylcarbazide method. Hexavalent Chromium was estimated by the acidic reaction with the 1,5-diphenylcarbazide method using a spectrophotometer (UV – Vis) at 540 nm. The soil samples were serially diluted and plated onto potato dextrose agar medium by spread plate method. The plates were then incubated at room temperature (30 -35ºC) for 48 hours. The identical colonies from each plate were inoculated onto fresh PDA plates to obtain pure cultures. Each isolate was characterized and further analyzed for heavy metal removal and tolerance studies.

2.2 Fungal Identification

Identification of the isolated fungal strains was made by observing the colony morphology on PDA plates and characteristics of spores by performing Lacto phenol cotton blue mounting with the pure culture of the isolated strain. Further, the organisms are confirmed by PCR-based identification using primer against the internal transcribed spacer (ITS) region of the 18S-rDNA sequence. Amplification of DNA extracted from the fungi was done by the method proposed by Zhao et al. (2001) with slight modifications in a Master cycler programmed for a first denaturation step of 5 minutes at 95ºC, followed by second denaturation (30 seconds at 95ºC), annealing (58ºC for 30 seconds) and primer extension (72ºC for 1 minute, for 40 cycles) with final extension for 10 minutes, at 72ºC.

2. 3 Estimation of Chromium Tolerance

Determination of fungal tolerance to Cr (VI) was carried out by agar gel diffusion method using different concentrations (10 – 1000 mg Cr (VI) / L) by dissolving the required quantities of potassium dichromate in distilled water. The concentrations of Chromium, *i.e.,* 10, 100, 500, and 1000mg Cr (VI) /L, were loaded in wells on PDA plates and were inoculated with respective fungi by swab culturing in triplicates. A control, without the metal solution, was kept for each plate. Plates were incubated at room temperature (24-30ºC) for 48 hours and were observed for clear zones around the colonies.

2.4 Bio-sorption studies

The bio-sorption ability of the fungal isolates was analyzed by inoculating each fungus into 100ml conical flasks containing potato dextrose broth amended with different concentrations (10mg/L to 1000mg/L) of Cr (VI). The initial concentration of Cr (VI) during inoculation was determined by the 1-5-diphenylcarbazide method. Optical density was measured at 540 nm using a spectrophotometer. The inoculated flasks were incubated at room temperature for ten days. The concentration of Cr (VI) was determined at each stage of incubation by recoding the Optical density at an interval of 24 hours, starting from Day1 to Day 10. After the incubation period, the residual Cr (VI) was determined the percentage of Cr (VI) removal ability of fungi at each concentration is calculated by using the removal isotherm formula.

$$n = [(Ci - Ce) / Ci] \text{ X } 100$$

Where 'Ci' = initial heavy metal level in solution (mgL^{-1}), 'Ce'= heavy metal level remaining in solutions after removal (mgL^{-1}). To evaluate the metal removal capacity of the biosorbent, "*n*" is used as the removal rate (%). Tests were conducted with initial heavy metal levels of 100 mg/L. Microorganisms can survive and grow in a range of pH. However, their activity will be maximum at an optimum pH. The fungal isolates were inoculated in a range of pH in triplicate from 2 to 10 along with Potassium dichromate (1000mg/L) and were incubated on a shaker incubator (120rpm) for 24 hours. Temperature also plays a key role in the growth and survival of microorganisms and also in their biochemical processes. The fungal isolates were inoculated into a series of 250 mL conical flasks containing potassium dichromate (1000 mg/L) in triplicate. The flasks were incubated at different temperatures (25, 30, 35, 40, and 45°C) and kept in an orbital shaker (120rpm) for 24 hours of incubation. The Chromium removal was measured as per the formula mentioned above. The fungal growth was determined by weighing the dry mycelium. Biomass from the broth was separated using a pre-weighed Whatman filter paper and kept for drying in a hot air oven overnight, and then the dry mycelial weight was determined. The fungal strain with the maximum Cr (VI) removal isotherm was used for mutation studies to estimate the metal removal ability of mutated strains. Potato Dextrose Agar plates were inoculated with the organism and were exposed under UV light for three different time intervals i.e., 5, 10, and 15 Minutes. The plates were removed from the UV light after the respective duration of exposure and incubated at room temperature for 48 hours. The UV-exposed fungal strain was inoculated into 100ml conical flasks containing potato dextrose broth amended with dif-

ferent concentrations (10mg/L to 1000mg/L) of Cr (VI). The initial concentration of Cr (VI) during inoculation was determined. Thus, the inoculated flasks were incubated at room temperature. The study was conducted for a period of 1- 10 days. The concentration of Cr (VI) was determined at each stage of incubation by recoding the OD readings on a daily, starting from Day1 to Day10. After the incubation period, the residual Cr (VI) was determined by the 1-5-diphenylcarbazide method. Thus, the percentage of Cr (VI) removal by mutated fungal strain was represented in a standard graph and calculated using the removal isotherm

3. RESULTS & DISCUSSION

3.1 Initial screening

The Chromium content of the collected soil samples was determined spectrophotometrically at 540nm, was found that the concentration ranged from 10 and 100mg/L. Sample 1 contained least concentration (10 mg/L) while 5 had 100mg/L. The rest of the samples T2 to T 4 showed concentrations of 18, 28, and 48 mg/L (Table 1).

Table 1: Concentration of Chromium in Collected Soil Samples

Soil Sample	Concentration of Cr (VI) mg/L
T_1	10
T_2.	18
T_3	28
T_4	48
T_5	100
Control	00

Identification of the isolated fungal strains was carried out by observing the phenotypic and culture characteristics on Potato dextrose Agar plates (Figure 1) and by performing Lactophenol cotton blue mounting with the pure culture of the isolated strain (Table 2). These were further confirmed with the ribotyping.

Figure 1: Fungal isolates from soil sample on PDA plates.

3.2. Identification of Fungal tolerance to Chromium by Agar gel diffusion method

The Agar gel diffusion method was performed on PDA plates to check the resistance of the isolated fungal strains to different concentrations of Cr (VI). It was found that the fungal isolates KJCFC3 and KJCFC7, i.e. *Penicillium chrysogenum* and *Paecilomyces lilanicus* showed maximum resistance up to 1000mg/L concentration of Chromium. *Aspergillus niger* and *Aspergillus terreus* were resistant up to 500mg/L but showed a zone of 32mm and 27mm diameter respectively. Whereas, *Aspergillus flavus, Cladosporium herbarum*, and *Trichoderma viridae* showed resistance only up to 100mg/L, and a clear zone of 29mm, 25mm, and 23mm respectively for the concentration 500mg/L and a clear zone of 37mm, 43mm and 40mm respectively for the concentration of 1000mg/L. Removal isotherm of Chromium also was calculated for all the fungal strains inoculated with concentrations of 10mg/l to 1g/L of Chromium in the medium (Table 3 & Figure 2).

The bio-sorption ability of each fungus was studied by determining the concentrations of Cr (VI) using a Spectrophotometer at 540 nm. The concentrations of Chromium were determined separately during the incubation period of 10 days. The first-day reading is the initial concentration of Cr (VI). The result revealed that there was a lag period of the initial three days as there was no growth and decrease in the concentration of Chromium in the medium. Thereafter, the fungal strains in the medium started growing, and there was a decrease in the concentration of Chromium in the medium. From the eighth day, it was noticed that there was no decrease in concentration, and thus the concentration remained constant. It was also noted that the maximum percentage of removal was seen in the flask containing the least concentration of Cr (VI), 10mg/L. The percentage of removal by the organisms KJCF1- 6 was 50.2, 60.1, 66.4, 80.2, 40.3, 60.6, and 100%, respectively, and this was due to the maximum growth of the organism at this concentration. The percentage of removal decreased with the increase in concentration, and all the organisms, except KJCFC 4 and 7, were able to remove the metal only up to the concentration of 600mg/L and beyond which there was no removal. The removal ability of KJCFC1 was seen only up to the concentration of 400mg/L, and the percentage of removal at this concentration is 1.92%. The removal of Cr by KJCFC3 was seen up to the concentration of 800mg /L, and the percentage of removal at this concentration was 0.53%. Even though the rate of removal gradually decreased beyond the concentration of 100, it showed removal even up to the maximum concentration of 800mg /L, i.e., 0.53%. The maximum removal by KJCFC4 was at the concentration of 10mg/L (80.2%), and the least removal of 1.04% was recorded at the concentration of 1000mg/L. The least removal of Chromium was observed with KJCFC5 in all the concentrations. The range was recorded as 40 to 3.8 % with 10 and 400 mg/L, respectively. Among all the fungal species, KJCFC7 showed a 100% removal rate at the concentration of 10mg/L, and then the percentage of removal decreased with the increase in concentrations. But there was removal even at the maximum concentration of Cr (VI) of 1000mg/L i.e., 1.05% (Table 4). Thus from this study, it is evident that the organism KJCFC7 showed the maximum removal tolerance to the heavy metal

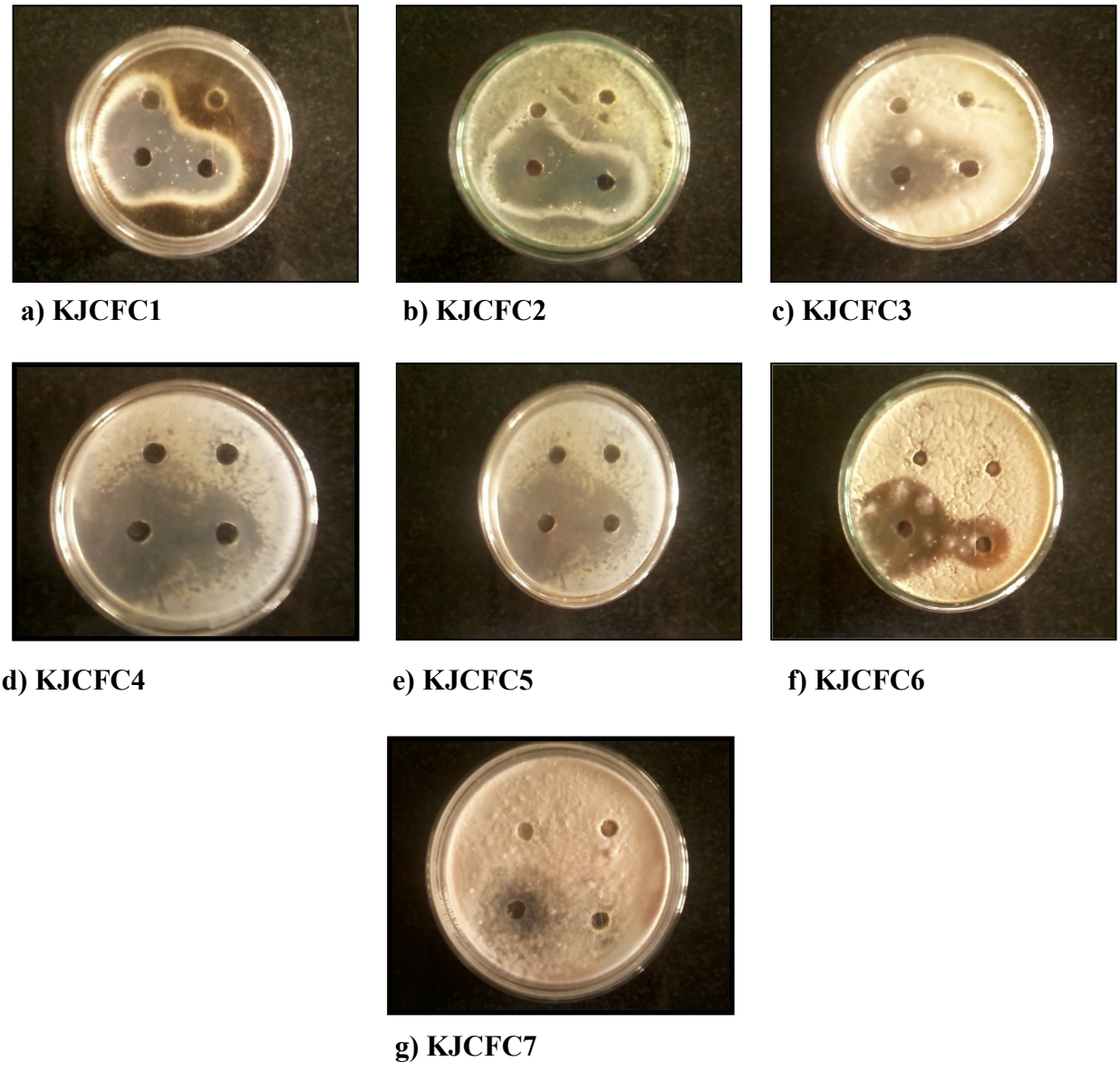
Table 2: Morphological Features of the Isolated Fungal colonies

Organism	Morphological features	
	Macroscopic	Microscopic
KJCFC1	The colony is a dense layer of dark-brown to black conidial heads. 	Hyphae are septate and hyaline. Conidiophores are 900-1600 µm long, smooth-walled, and terminate in pale-brown coloured globose vesicles 40-60 µm in diameter. Each vesicle is entirely covered with biseriate phialides that emerge from brown metulae. The phialides undergo blastic basipetal conidiogenesis to produce black globose mitospores 3-5 µm in diameter. *Aspergillus niger*
KJCFC2	The colony is yellow to dark yellowish-green, consisting of a dense felt of conidiophores or mature vesicles bearing phialides over their entire surface. 	Hyphae are septate and dichotomous, 45^0 angle branching. Conidial heads radiate to loosely columnar with age. Conidiophores are coarsely roughened, uncoloured, 400 to 850 µm long and 15–20 µm wide, vesicles spherical (20–45 µm), metulae (8–10 x 5–7 µm) covering nearly the entire vesicle in biseriate species. Conidial heads radiate. Conidia are pale green and conspicuously echinulate, smooth to very finely roughened, (sub) spherical, 3.5 - 6 µm in diameter. *Aspergillus flavus*
KJCFC3	Flat, filamentous, and velvety, woolly, and cottony in texture. The colonies are initially white and become blue-green, grey-green, olive-grey 	Septate hyaline hyphae (1.5 to 5 µm in diameter), simple/ branched conidiophores, metulae, phialides, and conidia are observed. Metulae is secondary branches that form on conidiophores. The metulae carry the flask-shaped phialides. The organization of the phialides at the tips of the conidiophores is very typical. *Penicillium chrysogenum*

KJCFC4	Colonies are yellowish-brown to cinnamon brown, consisting of a dense felt of conidiophores 	Conidiophore stipes smooth-walled hyaline. Conidial heads are loosely columnar. Vescilces subspherical, 10-20 micrometre diameter. Conidiogenous cells biserrate. Mutulae as long as the phialides. Conidia smooth walled, striate with SEM ellipsoidal, 1.5-2.5mm *Aspergillus terreus*
KJCFC5	Olivaceous-brown to blackish brown Cladosporium produces a black pigment so that when it grows on a surface, it looks black. Vegetative hyphae, conidiophores, and conidia are equally pigmented. 	Conidiophores are more or less distinct from the vegetative hyphae, are erect, unbranched Conidia are 1- to 4-celled, smooth, verrucose with a distinct dark hilum, and are produced in branched acropetal chains. The conidia closest to the conidiophore and where the chain branched are usually "shield-shaped". The presence of shield-shaped conidia, a distinct hilum, and chains of conidia are diagnostic features. *Cladosporium herbarum*
KJCFC6	Colonies are woolly and become compact in time. As the conidia are formed, scattered blue-green or yellow-green patches become visible. These patches may sometimes form concentric rings. The reverse is pale, tan, or yellowish 	Septate hyaline hyphae, conidiophores, phialides, and conidia are observed. Conidiophores are hyaline, branched, and may occasionally display a pyramidal arrangement. Phialides are hyaline, flask-shaped, and inflated at the base. They are attached to the conidiophores at right angles. The phialides may be solitary or arranged in clusters. *Trichoderma viridae*
KJCFC7	Colonies are flat, powdery, or velvety in texture and mature within three days. The surface colony colour is initially white becoming pink/violet. A sweet aromatic odour may be observed in older cultures 	Septate hyaline hyphae, conidiophores, phialides, conidia, and chlamydospores are present; Conidiophores are often branched and carry the phialides at their tips, and with sizes ranging from 3 – 4 x 400 - 600 µm; Phialides are thin, swollen at their bases, elongated at their tips are grouped in brush-like structures at the ends of the conidiophores. Conidia are oval/fusoid in shape, unicellular, hyaline to darkly coloured, smooth, and appear in long chains. *Paecilomyces lilacinus*

Table 3: Zone of Inhibition to Cr (VI) by fungal strains

Cr (VI) (mg/L)	Zone of inhibition (mm)						
	KJCFC1	KJCFC 2	KJCFC 3	KJCFC 4	KJCFC 5	KJCFC 6	KJCFC 7
10	No Zone	No Zone	No Zone	No Zone	No Zone	No Zone	No Zone
100	No Zone	No Zone		No Zone	No Zone	No Zone	
500	No Zone	29.1		No Zone	25.3	23.2	
1000	32.2	37.4	28.2	27.3	43.4	40.4	20.2



a) KJCFC1 **b) KJCFC2** **c) KJCFC3**

d) KJCFC4 **e) KJCFC5** **f) KJCFC6**

g) KJCFC7

Figure 2: Zones of Inhibitions observed signifying the Cr tolerance of the different fungi when grown on PDA plates containing Cr (VI) concentrations 100mg/L, 500 mg/L and 1000mg/L.

Cr (VI). Based on the results, the highest adsorption, zone of inhibition, and initial screening in a liquid medium, the fungal strain KJCFC7, was selected for further studies. The organism was identified as *Paecilomyces lilanicus* based on the colony morphology comparison and spore characteristics, and ribotyping.

3.3. Effect of pH on Cr (VI) removal by *Paecilomyces lilacinus*

The effect of hydrogen ion concentration in the medium affects the removal of Chromium by the organism. Different media with pH 2, 4, 6, 8, and 10 were inoculated with KJCFC7, and the removal was calculated after a duration of 7 days. The removal of Chromium was maximum at pH 4, and the percentage of removal was 11.57%. However, the rate of removal was reduced with the drop/increase in pH, i.e., at pH two and beyond six, the percentage of removal was seen at 10.52 and 6.31, respectively (Table 5). The result indicates the optimum pH for the growth of the organism and the acidic environment, which favours the removal of the metal ions.Most of the microbial surfaces are negatively charged due to the ionization of the functional groups, thereby contributing to the metal binding. The pH of the bio-sorption medium affects the solubility of the metal ions and the ionization state of functional groups. Fungal surfaces have a negative charge in the pH range of 2 to 6 *(Shankar et al., 2006).* The proton concentration is high at lower pH (<2), and heavy metal biosorption decreases due to the positive charge density on metal-binding sites, i.e. hydrogen ions compete effectively with metal ions in binding to the sites. The negative charge density on the cell surface increases with increasing pH due to deprotonation of the metal-binding sites. The metal ions then compete more effectively for available binding sites, which increases bio-sorption *(Kapoor et al., 1999).* The decrease in biosorption at higher pH (>6) is due to the formation of soluble hydroxylase complexes of the metal ions and their competition with hydroxyl ions for active sites. Beyond pH 8, precipitations of the ions as hydroxides occur *(Martins et al., 2006).*

3.4. Effect of temperature on Cr (VI) removal by the *Paecilomyces lilacinus*

Temperature is a critical environmental factor that affects the growth of organisms. A minimum of 25ºC and a maximum of 45ºC with 5-degree gradation were used for the incubation. The maximum removal of Cr (VI) was obtained at the temperature of 30°C, and the least percentage of removal was seen at temperatures 20 and 45, with a removal rate of 2.57 % and 2.06 %, respectively (Table 5). The temperature of the adsorption medium could be important for energy-dependent mechanisms in metal adsorption by microbial cells. Mostly adsorption is an exothermic process *(Martins et al., 2006).* In the case of exothermic bio-sorption processes, an increase in temperature has been found to reduce the bio-sorption capacity of the biomass *(Kumar et al., 2012; Suhasini et al., 1999).* This is attributed to the increase in temperature may increase metal desorption tendency from the interface to the solution (*Sari et al. 2007).* The fungal growth was optimum at the temperature of 30°C, which in turn was ambient for the fungal metal sorption.

3.5. Kinetics of Cellular Growth and Heavy Metal Removal

The fungal growth was determined by measuring the weight of dry mycelium. It was noted that the weight of the mycelium was maximum at the concentration of 10mg/L. The weight of the mycelium decreased with an increase in concentration as it couldn't grow well at high concentrations of Cr. The maximum growth obtained at 10mg/L showed the mycelial growth of 5.88 grams (Table 6).

3.6. The Biosorption ability of UV- Mutated Fungal Strain

The fungal species, *Paecilomyces lilacinus* which showed maximum tolerance and adsorption, was subjected to UV radiation in order to mutate the organisms. The time period of exposure was the duration of 5, 10, and 15 minutes. The mutated strain was further employed for its bio-sorption ability. It was found that the maximum percentage of removal obtained at 10mg/L concentration and the removal rate was 100% when the strain was mutated to 5 and 10 minutes, but the maximum percentage of removal obtained for the strain that was mutated for 15 minutes showed a decrease in removal rate up to 75.3% (Table 7).

Table 4: Removal Isotherm (%) of Hexavalent Chromium by Fungal Isolates

Cr(VI) mg/L	Removal Isotherm (%)						
	KJCFC1	KJCFC2	KJCFC3	KJCFC4	KJCFC5	KJCFC6	KJCFC7
C	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10	50.2	60.1	66.4	80.2	40.3	60.6	100
20	33.3	41.6	50.3	41.6	33.3	41.6	75. 4
40	30.3	30.4	40.4	30.5	35.0	35	44.5
60	21.4	28.5	33.3	28.5	28.5	28.5	37.3
80	12.9	12.9	25.6	12.9	12.9	12.9	12.9
100	05.88	05.88	11.4	11.7	11.4	11.7	10.2
200	4.31	04.31	7.9	7.9	7.9	7.9	10.07
400	1.92	01.92	3.8	5.12	3.8	5.7	8.9
600	0.59	0 .0	1.1	5.3	0.0	0.0	7.6
800	0.0	0.0	0.53	2.7	0.0	0.0	3.2
1000	0.0	0.0	0.0	1.04	0. 0	0.0	1.05

Table 5: Bio-sorption of Cr (VI) by *Paecilomyces lilacinus* (KJCFC 7) *at* pH and temperature

pH	Removal Isotherm (%)	Temperature (^{o}C)	Removal Isotherm (%)
2	10.52	25	8.24
4	11.57	30	10.82
6	6.31	35	7.21
8	5.26	40	3.09
10	3.15	45	2.06

Table 6: Rate of Chromium (VI) removal based on dry mycelial weight

Cr (VI) mg/L	Dry Mycelial Weight(g)
10	5.88
20	5.43
40	2.64
60	1.73
80	0.38
100	0.32
200	0.23
400	0.18
600	0.18
800	0.16
1000	0.16

Table 7: Rate of Biosorption of Cr (VI) by UV-mutated *Paecilomyces lilacinus* under different duration

Cr (VI) mg/L	Removal Isotherm of Chromium (%) versus duration of mutation (min)			
	5	10	15	Control
10	100	100	75.3	100
20	84.4	85.7	50.5	75.6
40	65. 5	65.4	40.2	44. 5
60	42.5	42.8	31.7	37
80	25.3	20.3	17.8	12.9
100	11.59	11.76	7.3	10.2
200	10.79	11.51	6.6	10.07
400	9.3	10.2	4.3	8.9
600	7.6	7.64	2.94	7.6
800	6.9	6.9	1.05	3.2
1000	6.25	6.25	0	1.05

4. CONCLUSION

The continuous search for a stable remediation process for the removal of contaminants from the environment has led to many findings. In such a study, hexavalent Chromium tolerant fungal species KJCFC1 to 7 were isolated from heavy metal contaminated environments and identified using morphological and microscopic observation followed by ribotyping. The organisms identified are three species of *Aspergillus, i.e., A. niger, A. terreus and A. flavus, Penicillium chrysogenum, Cladosporium herbarum, Trichoderma viridae, and Paecilomyces lilacinus*. From the above isolated fungal species, the most tolerant and effective bio-sorbent fungal species were sorted by bio-sorption studies, and their applicability in heavy metal removal from a simulated environment was evaluated at the laboratory scale. The most tolerant fungal species were identified to be similar to *Paecilomyces lilacinus*. The parameters like pH and temperature for both the growth and heavy metal removal were studied. The data revealed that *Paecilomyces lilacinus*, could tolerate Chromium (VI) toxicity up to 1000 mgL-1. The study also concluded that this particular fungus was able to completely remove the heavy metal Cr (VI) at the lowest concentration of 10mg/L, as the maximum growth and metal removal were also obtained at the concentration of 10 mg/L. The organism *Paecilomyces lilacinus* was also subjected to UV radiation – for mutation studies, and as a result, it was observed that the percentage of removal increased for the strain which was exposed to UV radiation for a duration of 10 minutes. The study demonstrated that the newly isolated heavy metal resistant *Paecilomyces lilacinus* from the metal-contaminated sites have potential application for the removal of Cr (VI), which comes out from various metal industries, especially the metal plating industry. Thus, *Paecilomyces lilacinus* can be used as an effective bio-sorbent to reduce the levels of toxic Cr (VI) in the environment and thereby bring down the level of heavy metal pollution.

REFERENCES

Alluri HK, Ronda SR, Settalluri V. S., Bondili JS, Suryanarayana V, Venkateshwar P (2007). *Biosorption: An eco-friendly alternative for heavy metal removal.* Afr. J. Biotechnol. 6: 2924-2931.

Ayele A, Haile S, Alemu D, Kamaraj M (2021). *Comparative utilization of dead and live fungal biomass for the removal of heavy metal: a concise review.* Sci. World J. 2021:5588111.

Bhainsa KC, D'Souza SF (1999). *Biosorption of uranium (VI) by Aspergillus fumigatus*. Biotechnol. Technique. 13: 695-699.

Bhattacharyya D, Cheng CYR (1987). *Activated carbon adsorption of heavy metals from single and multi-component systems.* Environ. Prog. 6: 110-118.

Christov LP, Van Driessel B, du Plessis CA (1999). *Fungal biomass from Rhizomucor pulsillus as adsorbent of chromophores from a bleach plant effluent*. Process Biochem. 35: 91-95.

Dhankhar R, Hooda A (2011). *Fungal biosorption- an alternative to meet the challenges of heavy metal pollution in aqueous solutions.* Environ Technol. 32(5-6): 467-91

Kumar RR, Lee JT, Cho JY (2012). *Toxic cadmium ions removal by isolated fungal strain from e-waste recycling*. J. Environ. Appl. Biores. 1 (1): 1-4

Muraleedharan TR. and Venkobachar, C. 1990. *Mechanism of biosorption of copper (II) by Ganoderma lucidum*. Biotechnol. Bioeng. 35: 320-325.

Oyewole OA, Zobeashia SSLT, Oladoja EO, Raji OR, Odiniya EE, Musa MA (2019). *Biosorption of heavy metal polluted soil using bacteria and fungi isolated from soil.* SN Appl. Sci. 1: 857

Padmavathy V, Vasudevan P and Dhingra SC (2003). *Biosorption of Nickel (II) ions on Baker's yeast.* Process Biochem. 38: 1389-1395.

Rostami KH, Joodaki MR (2002). *Some studies of cadmium adsorption using Aspergillus niger, and Penicillium austurianum, employing an airlift fermenter.* Chem. Eng. J. 89: 239-252.

Sari F, Braus GH, Irniger S (2007). *A process independent of the anaphase-promoting complex contributes to instability of the yeast S phase cyclin Clb5*. J. Biol. Chem. 282 (36): 26614-22

Sing, C. and Yu, J. 1998. *Copper adsorption and removal from water by living mycelium of white rot fungus Phanerochaete chrysosporium.* Water Res. 32: 2746-2752.

Statescu F, Cotiusca-Zauca D (2006). *Heavy metal soil contamination.* Environ. Eng. Manag. J, 5(5), 1205–1213.

Subudhi E, Ka RN (2008). *Rhizopus arrhizus-An efficient fungus for copper effluent treatment.* Int. J. Integr. Biol. 2: 166-170.

Suhasini IP, Sriram G, Asolekar SR, Sureshkumar GK (1999). *Biosorptive removal and recovery of cobalt from aqueous systems.* Proc. Biochem. 34: 239-247

Tereshina VM, Mar'in AP, Kosyakov NV, Kozlov VP, Feofilova EP (1999). *Different metal sorption capacities of cell wall polysaccharides of Aspergillus niger.* Applied Biochem. Microbiol. 35: 389-392.

Wuyep PA, Chuma AG, Awodi S, Nok AJ (2007). *Biosorption of Cr, Mn, Fe, Ni, Cu and Pb metals from petroleum refinery effluent by calcium alginate immobilized mycelia of Polyporus squamosus.* Sci. Res. Essays. 2: 217-221.

Yan GY, Viraraghavan T (2000). *Effect of pre-treatment on the biosorption of heavy metals on Mucor rouxii.* Water S. A. 26: 119-123.

Zhao J, Kong F, Li R, Wang X, Wan Z, Wang D (2001). *Identification of Aspergillus fumigatus and related species by nested PCR targeting ribosomal DNA Internal Transcribed Spacer Regions.* J Clin Microbiol. 39: 2261-2266.