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Research Article

Fungal biosorption of the heavy metals chromium(VI) and nickel from industrial effluent-contaminated soil

Shweta Yadav
Department of Environmental Science, Integral University, Lucknow- 226026 (U.P.), India
Anand Mishra*
Department of Environmental Science, Integral University, Lucknow- 226026 (U.P.), India

*Corresponding author. Email: anand.mishra.am@gmail.com

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Abstract
Heavy metals are ubiquitous contaminants that have accompanied man since the earliest ancient times, and unlike other environmental pollutants, they are chemical elements that man does not create or destroy. In the present study, the aim was to determine the biosorption potential of heavy metal-tolerant fungi that were isolated from compost soil samples contaminated by industrial effluents. The isolation was performed on potato dextrose agar (PDA) media supplemented with heavy metals. Chromium-Cr(VI) and nickel-Ni. The most dominant fungal species were found to be *Penicillium spp*. This fungus was screened for its ability to tolerate heavy metals by the plate diffusion and broth method and was highly tolerant to fungal species. The fungi were assessed for their ability to remove heavy metals from the culture media, and the culture conditions for the fungus were experimentally optimized. The isolated *Penicillium* species was found to show maximum growth at 35°C with media pH 6 for an incubation period of 168 hours. The isolate was able to tolerate 60-70 ppm concentrations of heavy metals under normal conditions. The ability of the isolate to take up metal was very effective, as after 96 hrs of incubation, it was capable of removing approximately 93.8% of Cr(VI) and 95.6% of Ni from the culture media, and complete uptake was observed after a 144 hr incubation period. The molecular characterization revealed the only isolate to be *Penicillium rubens* (Accession no. LC536286). The morphological characteristics of this fungus make it capable of biosorption of heavy metals, imparting its bioremediation potential and economic importance.

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

INTRODUCTION

In the present scenario, industrialization is growing increasingly globally, affecting the quality of water, food, feed, and weather (Haris *et al*., 2021). Various industries, such as the chemical, food, textile, and metallurgy industries, release a very high amount of waste, including toxic substances, to the environment. The chemical fertilizers and pesticides currently used in agricultural practices and the vehicles used for transportation discharge a large number of pollutants containing heavy metals into the environment (Sardar *et al*., 2013). Most importantly, food safety is considerably threatened by heavy metals. Various studies carried out on this topic have proven the accumulation of heavy metals in water (Ziaratie*t al*., 2016), rice (Yang *et al*., 2006), vegetables (*Makki et al*., 2014) and fish (*Sarma et al*., 2010). When these food samples are consumed, they 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 (Sardar *et al*., 2013). Many strategies have been used to solve the problems of heavy metal pollution in the environment. Applying 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. Microorganisms become the most acceptable microorganisms because they are easier to work with (*Jasrotia et al*., 2015). The most important advantage of microorganisms is their safety in human aspects. Due to excessive industrialization accompanied by rapid global population growth and an increase in agricultural practices, the heavy metals As, Cd, Cr (VI), Cu, Hg, Pb, and Zn are the most common environmental pollutants (He *et al*., 2015) and could have drastic effects on plants, such as stunned growth, necrosis and chlorosis in leaves and even plant death

(Rai *et al*., 2019).

The microorganism is capable of converting toxic heavy metals to nontoxic heavy metals through the process of metabolism (Lloyd, 2002). The methods involved in the bioremediation process by microorganisms are bio-oleaching, enzyme-catalyzed transformation, biomineralization, biosorption, and intracellular accumulation (Haferburg and Kothe, 2007). The maximum bioremediation of heavy metals occurs through the biosorption mechanism (Martin-Gonzalez *et al*., 2006). To obtain an efficient bioremediation process, 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 (Fu *et al*., 2012). Several studies have proven that active and lifeless fungal cells are capable of inorganic chemical adhesion (Tiwari *et al*., 2013). Penicillium spp. is known for its use in the conversion of toxic Cr (VI) to nontoxic Cr(III) by biosorption (Park *et al*., 2005) and has been reported in similar studies for its metal biosorption potential (*Oyewole et al*., 2019 and Xu *et al*., 2018). In terms of morphology, ecology, and metabolism, fungi adapt well according to environmental conditions and are responsible for carrying out the nutrient cycle and decomposition under natural conditions (Archana and Jaitly, 2015). 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 mycoremediation and involves the use of live or dead fungi for the removal of contaminants (Hamba and Tamiru, 2016). 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 (*Thenmozhi et al*., 2013). Mycoremediation is implicated as a successful method only when the correct identification and usage of the fungal species for the target heavy metal is performed. Fungal species are capable of accumulating heavy metals in their fruiting bodies in an efficient manner, hence making them unavailable or decreasing their concentration from the substrate they are growing on (Ogbo and Okhuoya, 2011). The future availability of heavy metals and other contaminants in the media depends upon the life of the fungi, chemical behaviour 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 such as hydroxyl, carboxyl, amino, sulphate, or phosphate and is mediated through interactions (Chen *et al*., 2020). The present study aimed to isolate and identify a heavy metal-tolerant fungal species and optimize its growth parameters to evaluate its biosorption potential.

MATERIALS AND METHODS

Sample collection and study area

The composite surface soil samples (not exceeding 5 cm depth) were collected from several sites of electroplating efflux-contaminated areas of Lucknow viz. Singh Auto Electrical Works, Sitapur Road (Purania), Saman Enterprises, Phool Bagh, Near Hussain Ganj, Pawan Batteries and Solar Appliances, Hussain Road, Rajaji Puram and Senta Battery house, Ring Road, Vikas Nagar, in sterile bags. The soil samples were transported to the Soil Laboratory of Integrated Biotechnological Research Institute (IBRI), Lucknow, for physio-chemical analysis. A small portion of the soil was stored at 4°C to ensure minimal biological activity, and fungus isolation was carried out within 24 hours of sample collection.

Isolation and Characterization of the fungi

To isolate 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 the spread plate technique, and plates were placed in an incubator at 28±1°C temperature. For morphological characterization, slides were prepared from the purified fungal isolates grown on PDA, stained with aniline blue stain and examined at 40X resolution. The identification of fungal isolates was made by comparing these morphological characteristics with those described by (Barnett, 1999).

Screening for selection of heavy metal-tolerant fungi

Fast-growing isolates with large biomasses were selected for screening of heavy metals (nickel and chromium). For this PDA, plates were prepared supplemented with 1 mM heavy metals. These heavy metal-containing plates were inoculated with a drop of spore suspension of isolates. The plates were incubated at 28±1°C°C for 168 hours (24 days). The effect of heavy metals on fungal growth 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 are the radial extension (cm) of the treated colony and untreated colony, respectively (Hassan *et al*., 2019).

Determination of heavy metal tolerance by fungal isolate

To determine the heavy metal tolerance capacity of the isolate, two different methods, plate diffusion and broth methods, were employed. PDA media were prepared and separately amended for the first method with variable concentrations of Ni and Cr(VI), viz., heavy metals such as $NiSO_4$ 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 isolate spotted in the centre and incubated at 28±1°C°C for 2-5 days, and then colony morphology and radial growth diameter were measured (Malik and Jaiswal, 2000). Tolerant fungi were studied by comparison among inoculated and control samples. For the latter method, fungal isolates were cultured in nutrient broth supplemented with variable concentrations of heavy metals (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 ppm). These sterile broths were inoculated with 0.1 ml of the fungal culture suspension and incubated at 28±1°C°C for 48 hours. After the incubation period, the optical density at 405 nm was measured against the media blank using a spectrophotometer (Varian Cary 50 UV–Vis double beam spectrophotometer) following the process described by Hassen *et al.* (1998).

Optimization of culture conditions

For the optimization experiment, three parameters, temperature, pH, and incubation period, were considered. The concentration of heavy metals was kept constant, and this selection was made based on the results 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 medium, three sets were prepared. From one set, different pH values of media were set in different tubes from pH 5-7 at an interval of 0.5 pH. These tubes were inoculated and kept at 28±1°C for 48 hours. From the second set, the pH was common, but after inoculation, the tubes were kept at a variable temperature from 25-45°C at an interval of 5°C. For the third set, different tubes were incubated for different time intervals. After the incubation period, the optical density of the samples collected from each tube of all three sets was taken at 405 nm to determine the optimum value.

Determination of heavy metal removal

The heavy metal removal from the culture solution was determined by the estimation of the residual amount of heavy metals left in the culture medium after 96 hours of the culture period, followed by the method described by *Shugaba et al*. (2011). The reduction in chromium ion concentration in media was indicated by the loss of yellow to orange color and by the quantitative decrease in Cr(VI) concentration in culture (Ackerley, 2004). For this, culture media supplemented with 50 ppm metal ions were inoculated separately by active and inactive mycelia of the fungus, and inactivation of mycelia was performed by autoclaving. Both were incubated under the same conditions for 4-5 days, and quantitative estimation of a small fraction of the media was filtered by membrane filters to remove the mycelia. The filtrate was used for quantitative measurement of heavy metal concentrations at specific time intervals.

Determination of total heavy metal uptake by fungal biomass

To determine the heavy metal uptake by fungal biomass, the fungal biomass was acid digested following the method described by Ksheminska *et al.* (2003). The mycelia were separated from the 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, kept at 30°C at room temperature (RT) for 30 minutes, and then heated gently on a heating mantle at 60°C for 30 minutes. The solution was then cooled at RT, and hydrogen peroxide was added at a ratio of 7:3 v/v HNO3/ H2O2 and again heated for 15 minutes. This mixture was centrifuged at 1500 rpm for 10 minutes, and the supernatant collected was used to determine the total chromium concentration using an atomic absorption spectrophotometer, AAS (Perkin Elmer, Japan Co, Ltd.), with a Zeeman graphite furnace.

Molecular characterization of the isolate

The isolated *Penicillium spp*. was then taken forward for molecular characterization by commercial DNA sequencing service sequencing of its 18S rRNA gene sequence. Later, the nucleotide sequence obtained was submitted to the National Center for Biotechnology Information (NCBI) sequence database available at https://www.ncbi.nlm.nih.gov. The online available program BLASTn was used to determine the related sequences with the taxonomic information in the databank at the NCBI website to accurately identify the species.

RESULTS AND DISCUSSION

The physio-chemical parameters viz., pH, electrical conductivity, organic carbon and matter, total dissolved solids and heavy metals of the effluent contaminated soil are given in Table 1. When the plates were observed after incubation, the culture media plates showed different kinds of fungal species, among them, one species was prominent and dominant both in the no. of colonies and in biomass. After the morphological characterization of the dominant colony, it was found to be from *Penicillium spp*. The morphological characteristics of the colony included elevated green colonies with circular margins having a woolly texture and septate hyphae with globose spores under a microscope. This fungus of *Penicillium spp*. was transferred to pure culture and was undertaken for further analysis of its biosorption potential in the study.

The selected fungal species was screened for its metal tolerance, and the tolerance index of this fungus ranged from 4-4.5 cm (diameter of the colony) for 25

Table 1. Physio-chemical characteristics of electroplating industrial effluent

Parameter	Mean 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 (VI)- 2.965 ± 0.55 (mg/L) Ni- 2.541 ± 0.45 (mg/L)

ppm $NiSO_4$ and 4-4.9 cm (diameter of the colony) for 25 ppm $K_2Cr_2O_7$. After this, the isolate was used to determine 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 60 ppm, showing visible growth, while visible growth was seen up to 50 ppm nickel. The colony diameter data of the metal tolerance capacity of *Penicillium spp*. is shown in Table 2.

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 metals, which is the maximum concentration at which visible growth was observed. The optical density of the three optimized parameters viz. incubation period, incubation temperature and medium pH was used to determine the fungal biomass concentration. The results of optimization showed that the selected *Penicillium spp*. had maximum growth at 35°C with media pH at pH 6 and an incubation period of 168 hours. The results are summarized in Table 3.

The fungal isolate that is the *Penicillium spp.* was assessed for its ability to remove heavy metals from the culture media within a certain period. The changes in the concentration of both heavy metals, i.e., Cr(VI) and Ni were efficiently reduced every 24 hours. At 96 hours, the media exhibited a Cr(VI) concentration of only 1.09 ± 0.12 ppm and a nickel concentration of 1.53 ± 0.21 ppm. Thus, with an 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.*

The isolated *Penicillium spp*. showed complete removal of both heavy metals from the culture media after 144 hours of incubation. This suggests the potential of the isolated fungal species in heavy metal removal, highlighting their environmental importance. The isolated fungal species were then taken forward for molecular characterization by 18S rRNA sequencing. The sequencing results 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 of NCBI.

The results of screening for the biosorption of heavy metals by the isolated *Penicillium spp*. revealed its potential. Its ability to do so may come from its inherent physiological characteristics, such as the cell wall. The *Penicillium* spp. has a rigid cell wall made up of polysaccharides such as chitins and glucans due to the surface-to-volume ratio, which helps these fungi absorb heavy metals such as cadmium into their cell walls (Chaney *et al*., 2007). *Penicillium spp*. are capable of releasing some extracellular enzymes, such as laccases and metal-binding proteins, that act as chelators capable of binding heavy metals and facilitating their absorption by the cell wall. The isolated *Penicillium spp.* can survive at high cadmium concentrations. A similar finding was reported that the *Penicillium notatum* isolated from the polluted stream near the industrial area of La plata (Argentina) was able to grow and remove up to 100-fold higher cadmium levels after 13 days of incubation (Leitao, 2009).

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, suggesting that pH above or equal to 7 is good for fungal growth and biosorption potential. The fungus *Penicillium* canescence was described as removing Cd,

Table 2. Showing the radial growth of *Penicillium spp*. against 0-70 ppm 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 = 8-9 cm (++++), Moderate growth = (7-5 cm) +++, Slightly growth = (4-3 cm) ++, Very slightly growth = 2-1 (+), No growth = 0 cm

Table 3. Optimization of *Penicillium spp.* under different growth parameters.

Optimization of temperature		
S. No.	**Temperature**	**O.D. at 405 nm**
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 405 nm**
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 405 nm**
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

Pb, Hg, and As ions from aqueous solutions by biosorption (Say and Yilmaz, 2003). The use of *Penicillium* chrysogenum to remove metal ions with high efficiency was first reported by *Niu et al*. (1993). 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 polyaromatic hydrocarbons and 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* (Boonchan *et al.,* 2000). The ortho-pathway degrades phenolic compounds, with catechol being the first intermediate (Leitao *et al.,* 2009). *Penicillium simplicissimum* was capable of effectively degrading 8.5 mM phenol used as the sole carbon source after 22 days of the incubation period. The catabolism of phenol produced catechol, hydroquinone, and *cis, cis*-muconic acid (Marr *et al*., 1996). The fungal isolate *P. notatum* showed the highest biosorption rate for cadmium at 10 ppm, at 77.67% (Oyewole *et al*., 2019). The accidental discovery of *P. simplicissimum* and subsequent studies conducted on this species revealed that this species is able to tolerate high concentrations of metals up to 1000 mg L−1 lead and chromium and 4500 mg L−1 Aluminium (Chen *et al*. 2020). Thus, there is an environmental importance of *Penicillium spp.* in the bioremediation process and the versatility of the species against various kinds of pollutants. In the present study, the Cr(VI) and Ni removal potential of *isolate P. rubens* can be used to effectively remove heavy metals from contaminated soil by optimizing the growth conditions for increased biomass over a short time span.

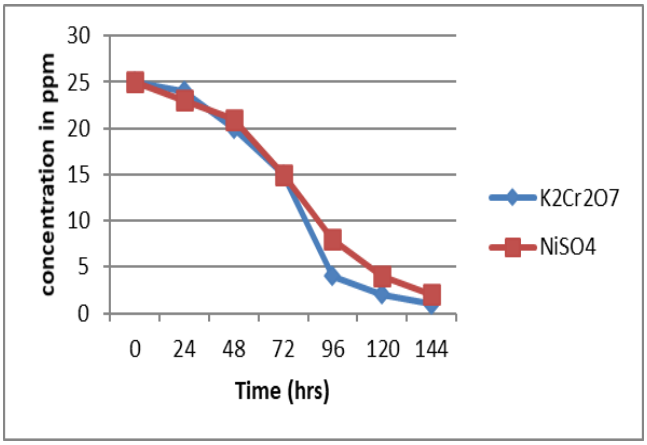

Fig. 1. *Profiles of Cr(VI) and Ni conc. during fungal growth in culture solutions treated with an initial concentration of 25 ppm*

Conclusion

The present study depicted the importance of the isolated *Penicillium spp*. and identified the isolate as *Penicillium rubens* (LC536286). This fungal species was found to potentially remove heavy metals – Cr(VI) and Niby biosorption. The ability of the fungus increases its economic importance, and its characteristics make it suitable for use in environmental cleanup. Thus, this species can be considered a good option for the bioremediation of other heavy metals. The study can be carried out to assess the capabilities of this fungal species on other pollutants so that the versatile nature of this fungus can be revealed.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Ackerley, D. F., Gonzalez C. F., Keyhan M., Blake, R. & Matin, A. (2004). Mechanism of chromate reduction by the Escherichia coli protein, NfsA, and the role of different chromate reductases in minimizing oxidative stress during chromate reduction. *Environmental Microbiology*, *6*(8), 851-860.
2. Archana, A. & Jaitly, A. K. (2015). Mycoremediation: utilization of fungi for reclamation of heavy metals at their optimum remediation conditions. *Biolife*, 3(1), 77-106.
3. Barnett, H. L. (1999). Illustrated genera of imperfect fungi/Hl Barnett and Barry B. Hunter (No. QK625.

A1. B3713 3A ED.). Burgess. Minneapolis, Minnesota. 1972.3. ed.
4. Boonchan, S., Britz, M. L. & Stanley, G. A. (2000). Degradation and mineralization of high-molecular-weight polycyclic aromatic hydrocarbons by defined fungal-bacterial cocultures. *Applied and Environmental Microbiology*, *66*(3), 1007-1019.
5. Chaney, R. L., Angle, J. S., Broadhurst, C. L., Peters, C. A., Tappero, R. V. & Sparks, D. L. (2007). Improved understanding of hyperaccumulation yields commercial phytoextraction and phytomining technologies. *Journal of Environmental Quality*, *36* (5), 1429-1443.
6. Chen, S. H., Cheow, Y. L., Ng, S. L. & Ting, A. S. Y. (2020). Bioaccumulation and biosorption activities of indoor metal-tolerant *Penicillium simplicissimum* for removal of toxic metals. *International Journal of Environmental Research*, *14*(2), 235-242.
7. Fu, Y. Q., Li, S., Zhu, H. Y., Jiang, R.& Yin, L. F. (2012). Biosorption of copper (II) from aqueous solution by mycelial pellets of *Rhizopus oryzae*. *African Journal of Biotechnology*, *11*(6), 1403-1411.
8. Haferburg, G., & Kothe, E. (2007). Microbes and metals: interactions in the environment. *Journal of Basic Microbiology*, 47(6), 453-467.
9. Hamba, Y. & Tamiru, M. (2016). Mycoremediation of heavy metals and hydrocarbons contaminated environment. *Asian J. Nat. Appl. Sci.*, *5*, 2.
10. Haris, M., Shakeel, A., Hussain, T., Ahmad, G., Ansari, M. & Khan, A. A. (2021). New trends in removing heavy metals from industrial wastewater through microbes. *Removal of Emerging Contaminants Through Microbial Processes*, 183-205. doi.org/10.1007/978-981-15-5901-3_26
11. Hassan, A., Pariatamby, A., Ahmed, A., Auta, H. S. & Hamid, F. S. (2019). Enhanced bioremediation of heavy metal contaminated landfill soil using filamentous fungi consortia: a demonstration of bioaugmentation potential. *Water, Air, & Soil Pollution*, 230(9), 1-20.
12. Hassen, A., Saidi, N., Cherif, M. & Boudabous, A. (1998). Effects of heavy metals on *Pseudomonas aeruginosa* and *Bacillus thuringiensis*. *Bioresource Technology*, *65*(1-2), 73-82.
13. He, Z., Shentu, J., Yang, X., Baligar, V. C., Zhang, T. & Stoffella, P. J. (2015). Heavy metal contamination of soils: sources, indicators and assessment. *J Envie Indicators*,9, 17-8.
14. Jasrotia, S., Kansal, A., & Mehra, A. (2017). Performance of aquatic plant species for phytoremediation of arsenic-contaminated water. *Applied Water Science*, *7*(2), 889-896.
15. Ksheminska, H., Jaglarz, A., Fedorovych, D., Babyak, L., Yanovych, D., Kaszycki, P. & Koloczek, H. (2003). Bioremediation of chromium by the yeast *Pichia guilliermondii*: toxicity and accumulation of Cr (III) and Cr (VI) and the influence of riboflavin on Cr tolerance. *Microbiological Research*, 158(1), 59-67.
16. Leitao, A. L. (2009). Potential of Penicillium species in the bioremediation field. *International Journal of Environmental Research and Public Health*, *6*(4), 1393-1417.
17. Lloyd, J. R. (2002). Bioremediation of metals; the application of microorganisms that make and break minerals.*Microbiol. Today.*29,: 67-9.
18. Makki, F. M. & Ziarati, P. (2014). Determination of histamine and heavy metal concentrations in tomato pastes and fresh tomato (*Solanum lycopersicum*) in Iran. *Biosci. Biotechnol. Res. Asia*, *11*(2), 537-544.
19. Malik, A. & Jaiswal, R. (2000). Metal resistance in Pseudomonas strains isolated from soil treated with industrial wastewater. *World Journal of Microbiology and Biotechnology*, 16(2), 177-182.
20. Marr, J., Kremer, S., Sterner, O. & Anke, H. (1996). Transformation and mineralization of halophenols by *Penicillium simplicissimum* SK9117. *Biodegradation*, *7*(2), 165-171.
21. Martin-Gonzalez, A., Díaz, S., Borniquel, S., Gallego, A. & Gutiérrez, J. C. (2006). Cytotoxicity and bioaccumulation of heavy metals by ciliated protozoa isolated from urban wastewater treatment plants. *Research in Microbiology*, 157(2), 108-118.
22. Niu, H., Xu, X. S., Wang, J. H. & Volesky, B. (1993). Removal of lead from aqueous solutions by Penicillium biomass. *Biotechnology and Bioengineering*, 42(6), 785-787.
23. Ogbo, E. M. & Okhuoya, J. A. (2011). Bioabsorption of some heavy metals by *Pleurotus tuber-regium* Fr. Singer (an edible mushroom) from crude oil polluted soils amended with fertilizers and cellulosic wastes. *International Journal of Soil science*, 6(1), 34-48.
24. Oyewole, O. A., Zobeashia, S. S. L. T., Oladoja, E. O., Raji, R. O., Odiniya, E. E. & Musa, A. M. (2019). Biosorption of heavy metal polluted soil using bacteria and fungi isolated from soil. *SN Applied Sciences*, 1(8), 1-8.
25. Park, D., Yun, Y. S., Jo, J. H. & Park, J. M. (2005). Mechanism of hexavalent chromium removal by dead fungal biomass of *Aspergillus niger*. *Water Research*, 39(4), 533-540.
26. Rai, P. K., Lee, S. S., Zhang, M., Tsang, Y. F. & Kim, K. H. (2019). Heavy metals in food crops: Health risks, fate, mechanisms, and management. *Environment International*, 125, 365-385.
27. Sardar, K., Ali, S., Hameed, S., Afzal, S., Fatima, S., Shakoor, M. B. & Tauqeer, H. M. (2013). Heavy metals contamination and what are the impacts on living organisms. *Greener Journal of Environmental*

Management and Public safety, *2*(4), 172-179.
28. Sarma, B., Acharya, C. & Joshi, S. R. (2010). Pseudomonads: a versatile bacterial group exhibiting dual resistance to metals and antibiotics. *African Journal of Microbiology Research*, *4*(25), 2828-2835.
29. Say, R., Yilmaz, N. & Denizli, A. (2003). Removal of heavy metal ions using the fungus *Penicillium canescens. Adsorption Science & Technology*, 21(7), 643-650.
30. Shugaba, A., Nok, A. J., Ameh, D. A. & Lori, J. A. (2011). Studies on some growth related changes in cultures of Aspergillus niger and *Aspergillus parasiticus* treated with hexavalent chromium and tannic acid. *International Journal of Biotechnology & Biochemistry*, 7(2), 251-265.
31. Thenmozhi, R., Arumugam, K., Nagasathya, A., Thajuddin, N. & Paneerselvam, A. (2013). Studies on mycoremediation of used engine oil contaminated soil samples. *Adv Appl. Sci. Res.*, *4*(2), 110-8.
32. Tiwari, S., Singh, S. N. & Garg, S. K. (2013). Microbially enhanced phytoextraction of heavy-metal fly ash amended soil. *Communications in Soil Science and Plant Analysis*, 44(21), 3161-3176.
33. Xu, X., Zhang, Z., Huang, Q., & Chen, W. (2018). Biosorption performance of multimetal resistant fungus Penicillium chrysogenum XJ-1 for removal of Cu2+ and Cr6+ from aqueous solutions. *Geomicrobiology Journal*, *35*(1), 40-49.
34. Yang, Q. W., Lan, C. Y., Wang, H. B., Zhuang, P. & Shu, W. S. (2006). Cadmium in soil–rice system and health risk associated with the use of untreated mining wastewater for irrigation in Lechang, China. *Agricultural Water Management*, *84*(1-2), 147-152.
35. Ziarati, P., Moslehishad, M. & Mohammad-Makki, F. M. (2016). Novel adsorption method for contaminated water by wild endemic almond: *Amygdalus scoparia*. *Biosciences Biotechnology Research Asia*, *13* (1), 147-153.