ORIGINAL ARTICLE

Bioaccumulation of chromium and nickel by fungal isolates from tannery effluent collection site from Kanpur, Uttar Pradesh, India

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Abstract

Aim: The aim of study was to analyze the physicochemical properties of tannery effluent and to isolate the promising fungi from heavy metals contaminated effluent to assess their metals accumulating abilities against chromium and nickel. **Materials and Methods:** The physiochemical parameters of tannery wastewater were analyzed. *Aspergillus niger* and *Penicillium* species were isolated and identified on the basis of their morphological and microscopic studies. The 6 heavy metals (Ni, Cu, Cr, Pb, Hg, and As) were analyzed through atomic absorption spectrometer from collected sample. Isolated fungal strains were assessed for their tolerance level against chromium and nickel heavy metals. Minimum inhibitory concentration was determined by well diffusion plate assays in Sabouraud dextrose agar medium. Bioaccumulation of chromium and nickel was determined on the basis of left metal ion concentration in aqueous medium. **Results:** Total dissolved solids, total suspended solids, and chloride were found to be 21300 mg/l, 1250 mg/l, and 740 mg/l, respectively. The pH and temperature of the wastewater were recorded to be 8.3 and 31°C, respectively. Dissolved oxygen (DO), biochemical oxygen demand, and chemical oxygen demand was found to be 2.72 mg/l, 3000 mg/l, and 3584 mg/l, respectively. The heavy metals such as nickel, chromium, copper, lead, mercury, and arsenic were found to be 0.280 mg/l, 0.478 mg/l, 0.006 mg/l, 0.0001 mg/l, 6.899 µg/l, and 0.790 µg/l, respectively. *Aspergillus niger* and *Penicillium* species were isolated and identified from the tannery effluent. *A. niger* tolerated 600 mg/l Cr and 100 mg/l Ni. *Penicillium* species tolerated up to 800 mg/l Cr and 400 mg/l Ni. Accumulation of *A. niger* at 25 mg/l aqueous medium of chromium was found to be 50.12%, at 50 mg/l 42.76%, at 100 mg/l 27.18% while *Penicillium* accumulation at 25 mg/l was 27.08%, at 50 mg/l 35.68%, at 100 mg/l 27.62%. In case of nickel accumulation of *A. niger* at 25 mg/l aqueous medium of nickel 66.64%, at 50 mg/l 37.70%, at 100 mg/l 34.47% while *Penicillium* accumulation at 25 mg/l 49.92%, at 50 mg/l 44.76%, at 100 mg/l 18.02%. **Conclusion:** The results of the study indicated the potential of these fungi in removal of heavy metals from industrial effluents containing higher concentration of heavy metals, namely, chromium and nickel. The further studies warranted to identify different fungi species capable of bioaccumulation of toxic heavy metals.

Key words: Atomic absorption spectrometer, bioaccumulation, fungi, heavy metals, tolerance level

INTRODUCTION

Globally, the incessant growth of human population has resulted into more demand for the life supporting commodities. Heavy metals are naturally occurring elements that have a high atomic weight and a density at least 5 times greater than that of water. Their multiple industrial, domestic, agricultural, medical, and technological applications have led to their wide distribution in the environment; raising concerns over their potential effects on human health and the environment.[1,2] Based on their high density, atomic weight, atomic number, and molecular weight heavy metals are characterized.[3] In industrial operations

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and manufacturing process huge water is consumed and discarded as an outflow which may be termed as effluent. The wastes and effluents which contains heavy metals flow out and emerge into the environment.[4] Copper, chromium, cadmium, and nickel are reported to be the most common heavy metals present in industrial wastes, and therefore, they are more widespread as contaminants in soil and water bodies.[5]

Tannery is the one of the oldest and fastest growing industry in India. There are about 2161 tanneries in India. Around 400 tanneries are in Kanpur, Uttar Pradesh. However, sustenance of tanneries is becoming increasingly difficult because of alarming level of environmental pollution caused by various tannery operations and practices. Tannery waste waters are mainly characterized by high salinity, high organic loading, and specific pollutants such as chromium.[6,7] The industrial effluent released directly or indirectly into natural water resources, mostly without proper treatment, poses a major threat to the environment. Kanpur city (India) is well known for tanneries and is also known for their pollution in the world due to the Tannery. In Kanpur, a large number of leather units are located at Jajmau area, right on the bank of river Ganga. These units, which use many toxic chemicals, are the single largest contributor to the pollution of the surface as well as groundwater of Jajmau area in Kanpur.[8] About 80% of the chemicals used in the tanning process are not absorbed by leather. Rest of these is released as waste, which is absorbed by bioaccumulation process in cultivated crops.[9] Chromium is widely used in tanning of the leather. It exists in two stable oxidation states Cr (III) AND Cr (VI). Cr (VI) is of particular concern as because of its high toxicity, it may cause many adverse effects on human health such as epigastric pain, hemorrhage, severe diarrhea, vomiting, and nausea, dermatitis by skin contact, ulcer, lung cancer, and tissue necrosis.[10] Nickel is a silvery white, hard, malleable, and ductile metal. It has a widespread distribution in the environment. The nickel maximum contamination limit standard is 0.2 mg/l. Drinking water generally contains nickel at concentration <10 µg/l. Nickel is known hematotoxic, immunotoxic, neurotoxic, genotoxic, reproductive toxic, pulmonary toxic, nephrotoxic, hepatotoxic, and carcinogenic agent.[11]

Biosorption can be an effective technique for the treatment of effluents containing heavy metals resulting from humans and industrial activities. Microorganisms including fungi can be explored for heavy metals removal because of their potential application in protection of environment and recovery of precious metals.[12,13]

Metal polluted environment contains fungi which have adapted to toxic concentration of heavy metals have become metal resistant. These fungi can be exploited for removal of toxic heavy metals form contaminated sources.[14] Therefore, it is required to isolate and screen heavy metal tolerant fungi. The present study attempts to isolate and screen heavy metal tolerant fungi and find out their efficiency to remove heavy metals from liquid media in *in vitro* conditions.

MATERIALS AND METHODS

Site and Sample Collection

Kanpur (Uttar Pradesh, India) is located at Indo-Gangetic plains between the parallels of 88°22′E longitude and 26°26′N latitude. About 400 tanneries are located at Jajmau (Kanpur). The tanning and other industries in Jajmau area, Kanpur are used to dump their all effluent in the common pump house where effluent is collected and further subjected for filtration. The water sample was collected from the pump house located at Jajmau in Kanpur city (Uttar Pradesh), India. A 5 Liters of effluent samples were collected in a clean container and stored in refrigerator (4°C) for fungal isolation and physicochemical analysis.

Analysis of Physicochemical Parameters

Standard sampling protocol was followed carefully to inhibit the intrusion of any foreign particles that may affect the results. Temperature was measured at the time of sample collection by mercury thermometer. A quantitative analysis for the determination of total suspended solid (TSS), total dissolved solid (TDS), total solid, sulfates, total alkalinity, carbonates, bi-carbonates, chlorides, and dissolved oxygen (DO) was carried out using simple laboratory methods. Electrical conductivity and pH of the effluent samples were measured by instruments. Biochemical oxygen demand (BOD) and chemical oxygen demand (COD) were determined by 3-day BOD test and closed reflux, titrimetric method, respectively. All analysis was performed using Bureau of Indian Standards method for water and wastewater.[15]

Isolation and Identification of Fungi

Fungi were isolated from the effluent sample by serial dilution method on potato dextrose agar (PDA) medium (HI MEDIA). Fungal colonies on PDA plates were identified based on their morphology and reproductive structural characteristics.[16] Isolated pure cultures were maintained on agar slants and stored in a refrigerator.

Determination of Heavy Metal Concentration through Atomic Absorption Spectrometer

Digestion of sample

The water sample was digested according to the APHA Standard method. 100 ml of water sample was taken in a beaker. 5 ml of HNO_3 was added. The solution was placed on to the hot plate under fume hood condition. When solution was vaporized remaining 10 ml residue, 100 ml volume was made up with deionized water in volumetric flask.[17]

Working standards

Cu, Cr, Pb, and Ni were detected on flame while As, Hg was detected on fluoroimmunoassays (FIAS). For flame analysis 0.5, 1, 3, 5, and 10 mg/l working standard of Cu, Cr, Pb, and Ni prepared and for FIAS analysis 20, 40, 60 µg/l of arsenic and 1, 3, 5, and 10 µg/l of mercury were prepared from stock solution of 1000 mg/l standard of metals. The instrument was calibrated with working standards and heavy metal concentration determined.[17]

Determination of Tolerance Level of Isolated Fungi against Cr and Ni

Plate diffusion assay was performed for determination of tolerance level of isolated strains. Zone of inhibition at particular concentration was considered as maximum resistance level of fungi. $K_2Cr_2O_7$ and $NiSO_4$ salts were used for metal concentration. Medium used was Sabouraud dextrose agar (HI MEDIA) with various concentrations of corresponding salts. 100-1000 mg/l range was analyzed with increment of 100 mg/l.[18]

Bioaccumulation of Heavy Metals by Fungal Isolates

The isolated fungi (*Aspergillus* spp. and *Penicillium* spp.) were assessed for their potential of bioaccumulation of targeted heavy metals. The different concentration of nickel and chromium were prepared using potassium dichromate and nickel chloride salts. 25, 50, and 100 mg/l concentration of chromium and nickel was applied. The stock solution of 1000 ppm was prepared and diluted by distilled water according to the concentration. Czapek Dox Broth medium (HI MEDIA) was used as growth medium. The final volume of broth adjusted according to the volume of the metallic salt solution added after autoclaving. 6 mm plugs of 7-day-old pure cultures of isolated strains were inoculated in Czapek Dox Broth with metallic salts. All inoculated flasks were incubated in orbital incubator shaker (Merck Genei) at 28°C for 7 days. The percent absorption was determined by the difference of initial metal ion concentration and left metal ion concentration.[19]

Estimation of Left Metal Ion Concentration in Filtrate through Atomic Absorption Spectrophotometer (AAS)

After the incubation mycelia was developed in the broths. The mycelia were filtered and digested. Metal-treated filtrate medium was digested using concentrated HNO_3 (5 ml). The content was boiled and evaporated to 10-15 ml on hot plate. Concentrated HCl (5 ml) was added and boiled till sample become clear and brownish fumes were evident. The concentration of heavy metals filtered solution was determined using AAS. The percent absorption was determined according to the left metal ion in the solution.[17]

RESULTS AND DISCUSSION

Analysis of the Characteristics of Tannery Wastewater

The characteristics of tannery wastewater vary considerably from tannery to tannery depending upon various factors such as chemicals used, amount of water used, and type of final product produced by a tannery. Typical characteristics of tannery wastewater are given in Table 1. The permissible limits for parameters in the wastewater from an industrial establishment mentioned in the rightmost column are stipulated by the World Health Organization.[20] Among the analyzed parameters, TDS and TSS were found 12040 mg/l and 1400 mg/l, respectively. pH and temperature of the wastewater were recorded to be 9.7 and 27°C. DO was found 1 mg/l. BOD and COD were found 3000 and 3584 mg/l, respectively.

Isolation and Identification of Fungi

A. niger and *Penicillium* spp. were isolated from the effluent. These two fungi were repeatedly observed on PDA agar plates. The fungi were identified on the basis of their characteristics [Figures 1 and 2].

A. niger colonies were black consisting of a dense felt of conidiophores. Reverse side was yellowish conidia brown, ornamented with warts, and ridges were shown. Hyphae were septate [Figures 1a and 2a].[16]

Table 1: Comparison of the parameters of the collected wastewater sample with the permissible limit stipulated by the WHO[20]

Parameters	Wastewater	Permissible limit
DO (mg/L)	1	4.5
TDS (mg/L)	12040	2100
TSS (mg/L)	1400	600
EC (µS/cm)	11033	1200
BOD (mg/L)	3000	30
COD (mg/L)	3584	250
Cl (mg/L)	740	1000
pH	9.67	5.5-9

DO: Dissolved oxygen, TSS: Total suspended solid, TDS: Total dissolved solid, EC: Electrical conductivity, BOD: Biochemical oxygen demand, COD: Chemical oxygen demand, WHO: World Health Organization

Penicillium spp. colonies surface at first appeared white then became light bluish green after 3 days. Reverse side was white. Septate hyphae with branched or unbranched conidiophores which have secondary branches known as medulla were detected. Entire structure formed a brush border [Figures 1b and 2b].[16]

Determination of Heavy Metal Concentration

Metals concentration detected in water sample by AAS and the results were presented in Table 2. Heavy metals content of sample analyzed by AAS showed the presence of higher concentrations of metals. Chromium is highly soluble in water and carcinogenic to human.[21] Ni (II) is more toxic and carcinogenic metal when compared with Ni (IV). Due to their toxic effects on living systems stringent limits have been stipulated for the discharge of chromium and nickel into the environment.[22] A higher concentration of mercury is a matter of concern, as it will accumulates in the kidneys, neurological tissue, and the liver. All forms of mercury are toxic and their effects include gastrointestinal toxicity, neurotoxicity, and nephrotoxicity.[23]

Tolerance Level of Isolated Fungi against Cr and Ni

Critical metal concentration limit was identified at 100-1000 mg/l (with increase of 100 mg/l). Zone of inhibition was observed at particular concentrations. *A. niger* tolerated 600 mg/l of Cr and 100 mg/l of Ni. *Penicillium* spp. tolerated 800 mg/l of Cr and 400 mg/l of Ni [Table 3, Figures 3 and 4].

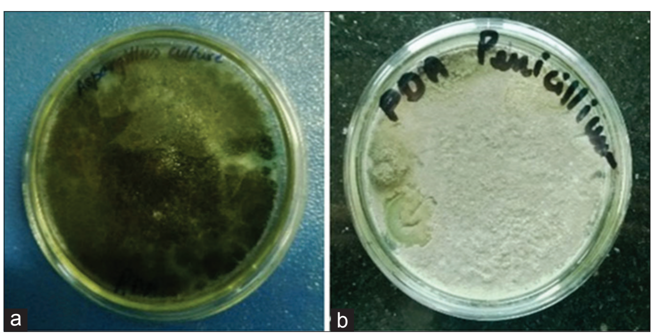

Figure 1: Plate cultures of identified fungal isolates. (a) *Aspergillus niger* and (b) *Penicillium* spp.

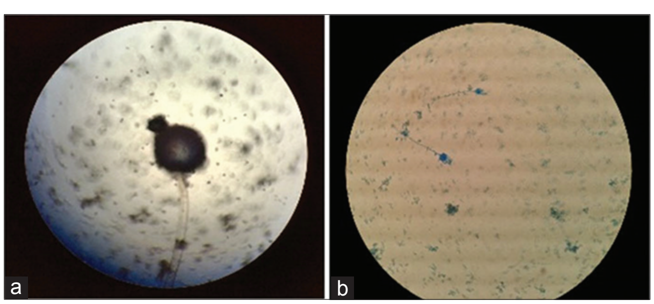

Figure 2: Microscopic view of fungal isolates. (a) *Aspergillus niger* and (b) *Penicillium* spp.

Metal tolerance of fungi belonging to metal pollutant habitat has been reported by other workers.[24,25]

Table 2: Different metal concentration in the water sample

Metals	Concentration
Ni	0.280 mg/l
Cu	0.006 mg/l
Cr	0.478 mg/l
Pb	0.0001 mg/l
Hg	6.899 μg/l
As	0.790 μg/l

Table 3: Results of plate assays of minimum inhibitory concentration

Fungus	Metal	Zone of inhibition (mg/l)
Aspergillus	Cr	700
Aspergillus	Ni	200
Penicilium	Cr	900
Penicilium	Ni	500

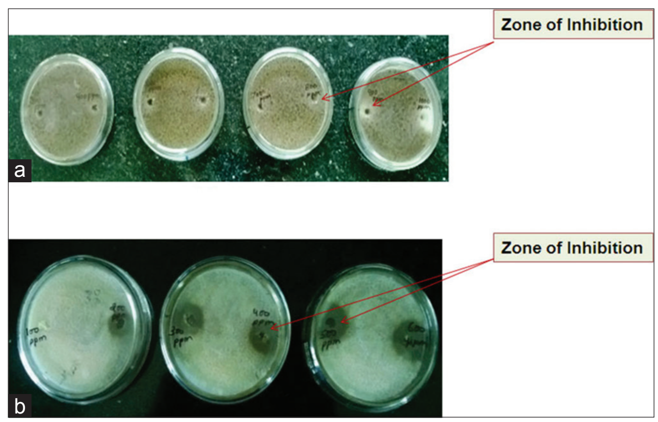

Figure 3: Plate assays of *Aspergillus* for maximum resistance level. (a) *Aspergillus* in aqueous concentrations of Cr (b) *Aspergillus* in aqueous concentrations of Ni

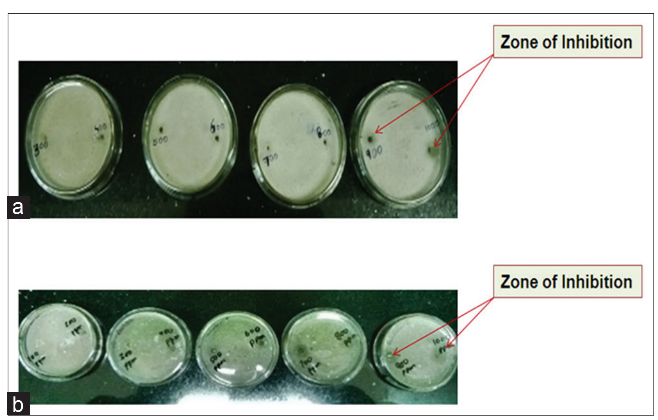

Figure 4: Plate assays of *Penicllium* for maximum resistance level. (a) *Penicillium* in aqueous concentrations of Cr and (b) *Penicillium* in aqueous concentrations of Ni

Estimation of Left Metal Ion Concentration in Broth Filtrate and % Absorption by Fungi

The different concentration of chromium and nickel against *A. niger* and *Penicillium* were represented in the Figures 5 and 6. In the present investigation, accumulation potential of heavy metals by *A. niger* at 25 mg/l aqueous medium of chromium was 50.12%, at 50 mg/l 42.76%, at 100 mg/l 27.18% while *Penicillium* accumulation at 25 mg/l was 27.08%, at 50 mg/l 35.68%, at 100 mg/l 27.62%. In case of nickel, *A. niger* accumulated at 25 mg/l aqueous medium of nickel 66.64%, at 50 mg/l 37.70%, at 100 mg/l 34.47% while *Penicillium* accumulation at 25 mg/l 49.92%, at 50 mg/l 44.76%, at 100 mg/l 18.02%. The uptake of chromium by A. niger was more significant in comparison to *Penicillium*. While uptake of Ni by *Penicillium* was more in comparison to *A. niger*. Similar results with respect to uptake of Ni and Cr by fungi have been reported earlier.[25-30]

CONCLUSIONS

The present study concludes that fungi isolated from tannery effluent have the ability to resist higher concentrations of chromium and nickel. Chromium and nickel are released into the environment by a large number of processes such as electroplating, leather tanning, wood preservation, pulp processing, and steel manufacturing. These two metals are of major concern because of their larger usages in developing countries and their non-degradable nature. The physical parameters studied in the effluent namely pH, total hardness, total suspended, and dissolved solids were at higher level. The chemical parameters, namely, DO, COD, BOD, and chloride, are found to be beyond the permissible limits (except chloride). From this study, it may be conclude that potential strains of fungi having the capacity of effective biosorption of heavy metals can be utilized for bioremediation of heavy metals as an eco-friendly measure.

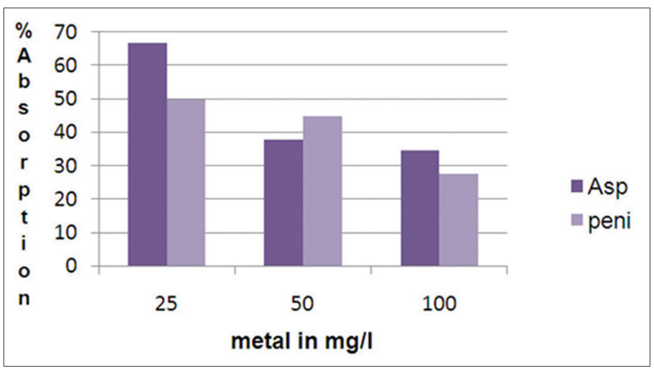

Figure 5: Chromium accumulation by *Aspergillus niger* and *Penicillium* spp. at 25, 50, and 100 mg/L metal concentration

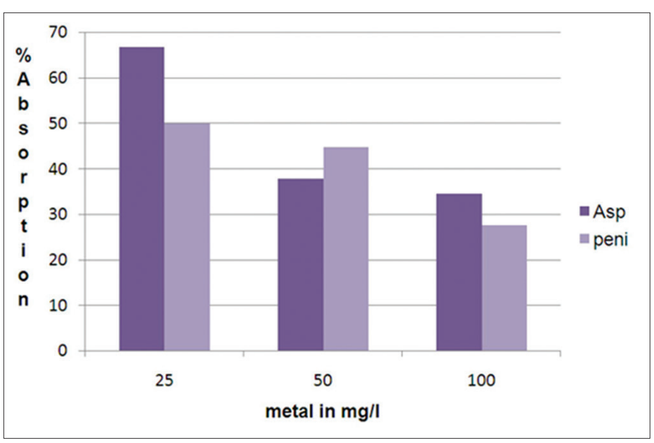

Figure 6: Nickel accumulation by *Aspergillus niger* and *Penicillium* spp. at 25, 50, and 100 mg/L metal concentration

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