



Bioremoval of Different Heavy Metals in Industrial Effluent by the Resistant Fungal Strain *Aspergillus niger*

K. J. Naveen Kumar*[†] and J. Prakash*

*Department of P.G. Studies and Research in Microbiology, Kuvempu University, Jnana Sahyadri, Shankaraghatta-577 451, Shivamogga District, Karnataka, India

[†]Corresponding author: K. J. Naveen Kumar; naviekj@gmail.com

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ABSTRACT

Developing countries are increasingly concerned with pollution due to toxic heavy metals in the environment. Unlike most organic pollutants which can be destroyed, toxic metal ions released into the environment often persist indefinitely circulating and eventually accumulating throughout the food chain thus posing a serious threat to mankind. The use of biological materials for heavy metal removal or recovery has gained importance in recent years due to their good performance and low cost. Among the various sources, both live and inactivated biomass of organisms exhibits interesting metal binding capacities. Their complex cell walls contain high content of functional groups like amino, amide, hydroxyl, carboxyl, and phosphate which have been implicated in metals binding. In the present study, *Aspergillus niger* was used to analyze the metal uptake from an aqueous solution. The determination of Cu^{+2} , Pb^{+2} , Cd^{+2} , Zn^{+2} , Co^{-2} and Ni^{+2} in samples was carried out by differential Pulse Anodic Voltammetry (DPASV) and the Voltammograms. Production of oxalic acid was carried out by submerged fermentation. The organism used in the present study has the ideal properties to sequester toxic metals and grow faster.

INTRODUCTION

Fungi are ubiquitous in nature and can be found in soil, sediments, and aquatic environments such as lakes, ponds, rivers, marine water, wastewater, industrial effluents, etc. They are heterotrophic organisms, mostly aerobic or microaerophilic in nature. Fungi exist in a variety of morphological and physical states, which makes it difficult to quantify and identify them by cultural techniques. Cultural methods for fungi are similar to those of bacteria but must be modified to inhibit bacterial growth. They can also be used for the recovery of the metals from ores or even other forms (Elizabeth & Priyadarshini 2004, Abdul & Sirajuddeen 2006, Acosta-Rodriguez et al. 2018).

Metals in general are a class of chemical elements that form lustrous solids, which are good conductors of heat and electricity. However not all metals fit this definition, for example, mercury is a liquid. Metals such as arsenic, boron germanium, and tellurium are generally considered metalloids or semimetals in that their properties are intermediate between metals and those of non-metals. The metals associated with metal pollution are arsenic (As), cadmium (Cd), copper (Cu), chromium (Cr), mercury (Hg), lead (Pb), and zinc (Zn). Toxic metals include those with no known biological function. These include argentinum

(Ag), cadmium (Cd), tin (Sn), mercury (Hg), tellurium (Tl), lead (Pb), aluminum (Al), and metalloids like germanium (Ge), antimony (Sb), and silicon (Si). The metalloids exert different toxic effects than metals because they have different chemistries (Akthar & Mohan 1995, Aneja 2001, Barros et al. 2003).

Metal pollution results when human activity disrupts normal biogeochemical activities or by the disposal of concentrated metal wastes. Sometimes a simple metal is involved, but more often a mixture of metals is present (Bishnoi et al. 2004, Chatterjee et al. 2006). Mining ore refinement, nuclear processing, and the industrial manufacture of batteries, metal alloys, electrical components, paints, preservatives, and insecticides are some of the examples of processes that produce metal by-products. Examples of specific metal contaminants include copper and zinc salts that are extensively used as pesticides in agricultural settings, silver salts that are used to treat skin burns, lead which is utilized in the production of batteries, cable sheathing pigments, and alloys (Khasim Beebi et al. 1999, Hussain et al. 2004, Kumar & Selvisabhanayakam 2006). Other examples include mercury compounds that are used in electrical equipment, paints, thermometers, fungicides, as preservatives in pharmaceuticals and cosmetics, and triorganotin compounds such as tributyltin chloride and triphenyltin chloride, which can be

used as antifouling agents in marine paints because of their toxicity to plankton of bacteria (Mani & Mohini 2005).

Thus, while metals are ubiquitous in nature, human activities have caused metals to accumulate in the soil. Such contaminated soils provide a metal sink from which surface waters, groundwaters, and redox zone can become contaminated. Metal contamination has occurred for centuries since metals have been mined and used extensively throughout human history. Atmospheric metal concentration has also increased. Contaminated soil contributes to high metal concentrations in the air through metal volatilization. Several methods have been employed for the detection of metals (Naseem Akhtar & Mohan 1995, Essoka & Umaru 2006). One among them is stripping voltammetry, among the electroanalytic techniques, differential pulse anodic stripping voltammetry is the most sensitive and suitable for detection of the heavy metal ions like cadmium, copper, zinc, lead, etc., because of low cost and easy operation (Asha & Juwarkar 1988, Acharya et al. 2004).

Bioleaching is a simple and effective technology for metal extraction from low-grade ores and mineral concentrates. The process involves the transformation of solid compounds into soluble and extractable elements which can be recovered. It represents a 'clean technology' with low cost and low energy consumption as compared to conventional methods. Metal recovery from sulfide minerals is based on the activity of chemolithotrophic bacteria, mainly *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans* which convert insoluble metal sulfides into soluble metal sulfates (Esposito et al. 2001, Fawzy et al. 2017). Non-sulfide ores and minerals can be treated by heterotrophic bacteria and by fungi. In these cases, metal extraction is due to the production of organic acids and chelating and complexing compounds excreted into the environment. At present bioleaching is used essentially for the recovery of copper, uranium, and gold and the main techniques employed are heap, dump, and in situ leaching. Tank leaching is practiced for the treatment of refractory gold ores (Ghaed et al. 2001, Hossain 2006). Bioleaching has also some potential for metal recovery and detoxification of industrial waste products, sewage sludge, and soil contaminated with heavy metals. The present work is undertaken with the following to study the bioleaching property of *Aspergillus niger*.

MATERIALS AND METHODS

Sample Collection

The industrial effluent samples were collected from the outlet five different times, near the bus stop Bhadravathi, Shimoga, and Karnataka using screw-capped plastic bottles

of 1.5 L capacity. The collected samples were preserved in the laboratory at room temperature.

Isolation of *Aspergillus niger* from Industrial Effluent by Serial Dilution Method

One mL of industrial effluent sample was taken in a test tube containing nine mL of sterile distilled water and shaken well in a vortex mixer. From this stock, various dilutions were prepared from 10^{-1} to 10^{-7} , using sterile distilled water. One mL of the diluted sample was poured into Petri plates containing the Potato Dextrose agar medium. Streptomycin was added to the molten medium after autoclave and the plates were incubated at $28 \pm 2^\circ\text{C}$ for 4 to 5 days to identify the fungi. Distinct fungal colonies grown on Potato Dextrose agar medium were isolated from repeated plating (Aneja 2001).

Identification of Fungi

Fungal morphology was studied macroscopically by observing colony features (color and surfaces) under a Stereo binocular microscope and microscopically by staining with Lacto phenol cotton blue and observed under binocular compound microscope for the conidia, conidiophores, and arrangement of spores (Funder 1961, Domsch 1980, Subramanian 1983).

Metal Detection

The sample was allowed to settle for a week. The liquid was decanted. The slurry was centrifuged for 15 min at 7000 rpm. The supernatant was decanted and the solid residue was collected and dried at $40\text{-}50^\circ\text{C}$ using the hot air oven. After drying, the residue was ground well using a pestle and mortar. The resultant mixture was weighed and preserved and a part of which was sent for metal analysis using stripping voltammetry (Schinner & Bungstaller 1989, Vijendra & Chandel 2001).

Processing for Bioleaching

The fermentation broth was prepared using glucose, peptone, malt extract, and double-distilled water. 200 mL of fermentation broth was poured into four different 250 mL conical flasks. The flasks containing media were sterilized at 121°C for 20 min. After cooling, the media was inoculated with *A. niger* at aseptic conditions. The flasks were then kept on the rotary shaker for 10 days at 120 rpm at 29°C . Titrations were carried out on the 10th day to estimate the oxalic acid production. 1.5 mg of the mixture was taken in the tea bag and suspended in the medium and left for bioleaching for 10 days (Satchanska et al. 2005).

Bioaccumulation Process

Metal used for analysis: Heavy metals such as zinc oxide and nanoparticles like nickel hydroxide, zinc hydroxide.

Preparation of stock solution: A stock solution of 500 ppm is prepared by dissolving 250 mg of the metal in 500 mL of double-distilled water. From this stock solution 20 ppm, 40 ppm, 60 ppm, 80 ppm, and 100 ppm concentrations were prepared.

Protocol for bioaccumulation process: Clean conical flasks were labeled with the metal concentration, organism to be inoculated, and the name of metal. 50 mL of sabouraud's dextrose broth was added. The respective metal concentration of 20 mL volume was added. Two loopful of the organism (*A. niger*) was inoculated. The flasks were incubated at room temperature for 7 days.

Metal detection: The residual metal in the aqueous solution was detected by using Atomic Absorption Spectroscopy (AAS). AAS is an instrument where the metal concentration in a solution is determined by aspirating a metal solution into an air acetylene flame to atomize the metal. A metal-specific lamp is placed into the AAS and is used to determine the differences in the light absorbance between a sequence source and the metal solution. The difference affects the amount of metal present.

Analysis of Complex Formation using UV Spectroscopy

After 10 days, the leachate and the control broth were made to undergo UV spectroscopy for the detection of complex formation. UV visible spectra of the controlled broth were recorded in water as a solvent. Then, 2 mL of the solution was taken and diluted with 2 mL of water (Jamil & Kumar

1997, Jyotsna 2003).

Processing: The incubated samples were filtered through Whatman No 1 filter paper to separate fungal mat and supernatant. The supernatant was decanted to another flask and the fungal biomass obtained was dried in a hot air oven at 250°C for 30 min. The supernatant obtained was subjected to a digestion process.

Digestion: To analyze the residual metal present in the supernatant, the supernatant was digested using concentrated nitric acid. The sample was digested in the following way. 100 mL of sample was taken in a beaker and 5 mL of concentrated nitric acid was added. The beaker was placed on a hot plate at 100°C. When the volume of the sample was reduced to 20 mL, then 5 mL of concentrated nitric acid was added. A Petri dish lid was placed on the beaker and evaporated to dryness. Then, 20-30 mL of distilled water was added, stirred well, and filter through Whatman No. 1 filter paper. Then the solution was made up to 100 mL by adding distilled water. The filtered solution obtained was subjected to atomic absorption spectroscopy (AAS) for the detection of residual metal.

Statistical analysis: All the results were statistically analyzed using SPSS software to determine the mean of three replicates and its standard error value from independent experiments.

RESULTS AND DISCUSSION

Isolation of *Aspergillus niger* from Industrial Effluent

Fungal morphology was studied macroscopically by observing colony features (color and surfaces) under a Stereo binocular microscope and microscopically by staining with

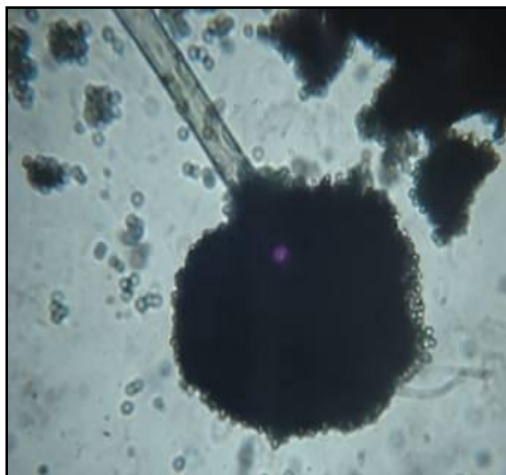
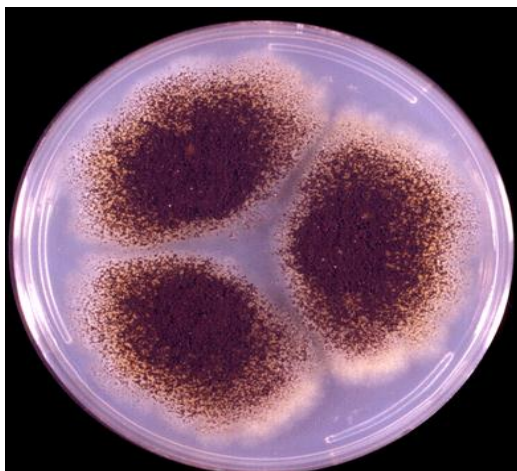


Fig. 1: Macroscopic and Microscopic view of *Aspergillus niger*.

Lacto phenol cotton blue and observed under a binocular compound microscope (Fig. 1).

Production of Oxalic acid by Submerged Fermentation Process

Production of oxalic acid was done by submerged fermentation process (Fig. 2).

Metal Detection

The processed sample was found to contain heavy metals like zinc, copper, cadmium, lead, cobalt, and nickel. The determination of Cu^{+2} , Pb^{+2} , Cd^{+2} , Zn^{+2} , CO^{-2} , and Ni^{+2} in the sample was carried out by Differential Pulse Anodic Voltammetry (DPASV) and the Voltammograms as shown in Fig. 3 and 4.

The results of the samples analyzed by DPASV were as follows, $\text{Cu} = 0.95 \text{ mg.kg}^{-1}$, $\text{Co} = 0.036 \text{ mg.kg}^{-1}$, $\text{Pb} = 0.50 \text{ mg.kg}^{-1}$, $\text{Ni} = 0.195 \text{ mg.kg}^{-1}$ and $\text{Zn} = 3.10 \text{ mg.kg}^{-1}$

Preliminary Work

Initially, we wanted to know the effect or binding capacity of the oxalic acid with the metals present in the industrial effluent sample. So, we started a preliminary test in which 10% oxalic acid (10 gm of oxalic acid in 100 mL distilled water) solution was prepared. To this, the processed sample was suspended using the teabag.

After two days the strength of the oxalic acid was estimated. Interestingly, it was found that the strength of the oxalic acid had decreased. Therefore, we allowed the experimental setup to stand for another five days. Finally,

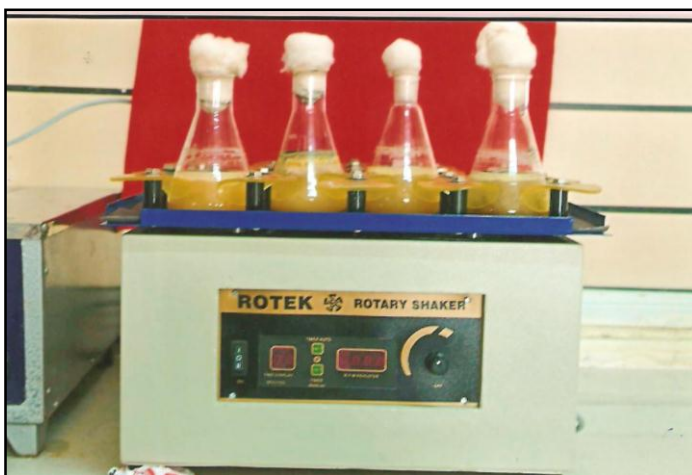


Fig. 2: Production of oxalic acid using malt extract broth by submerged fermentation process on a rotary shaker at 120 rpm.

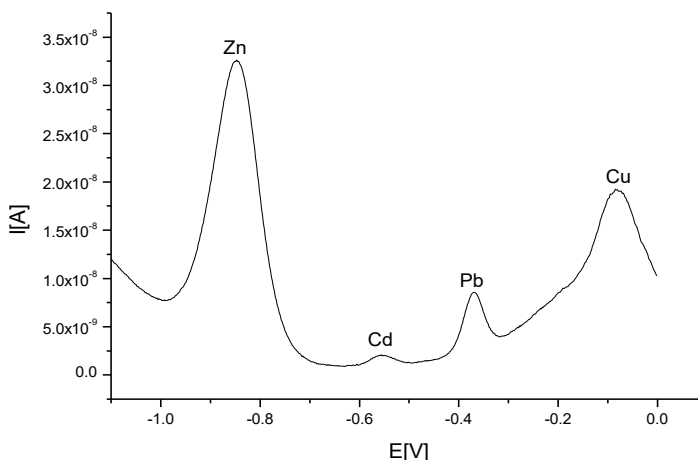


Fig. 3. Stripping voltammogram of the sample extracted from industrial effluent

Note: pH 2.25, Deposition potential -1.25 V, Deposition time 120 sec, modulation amplitude 20 mV, modulation time 0.5 sec, interval time 0.2 sec

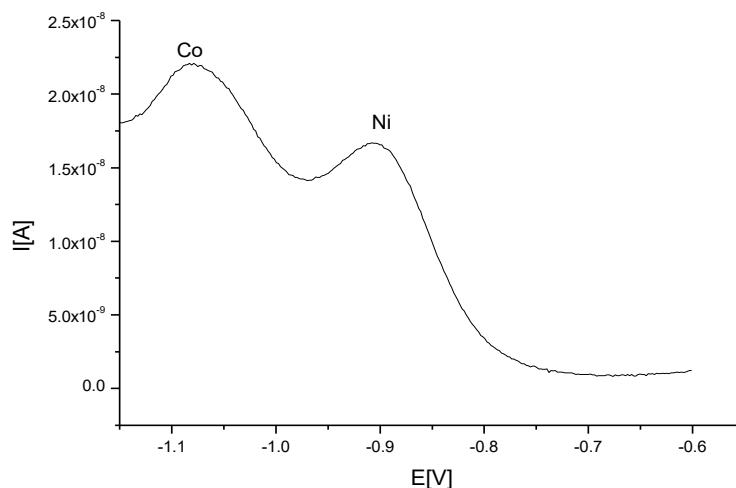


Fig. 4: Stripping voltammogram of the sample extracted from industrial effluent

Note: pH 9.2, Deposition potential -1.25 V, Deposition time 120 sec, modulation amplitude 20 mV, modulation time 0.5 sec, interval time 0.2 sec

after five days, final titration was carried out and it was observed that the strength of the oxalic acid had further decreased.

By observing the above results, fermentation broth containing glucose, peptone, and malt extract was used for the cultivation of *A. niger* and thereby oxalic acid production (Table 1).

Bioleaching

As expected, it was found that the strength of the fermented broth was decreasing. This was confirmed by the titrations performed (Table 2).

The decrease in the strength of the oxalic acid in the broth

confirmed the process of bioleaching. This was possible because more and more carboxylic (COO^-) groups formed complexes with the metal ions through coordination bonds. This gradually brought down the acidity of the broth, thereby indicating the progress of the bioleaching process.

After comparison, there was a decrease in controlled broth and leachate. In the control, it was due to fungal activity, whereas in leachate it was due to both fungal activity as well as bioleaching.

Detection of Complex Formation

The complex formation was further confirmed by UV spectrophotometry. UV visible spectra were recorded in Shimad-

Table 1: Oxalic acid production was estimated through titration assay.

Date	Sample taken	Trial No.	Initial reading	Final reading	Vol. of NaOH consumed	Strength of NaOH	Strength of oxalic acid
14-02-2019	Control	1	0	7.9	7.9	1	1.58
		2	7.9	15.8	7.9	1	1.58
	Leachate	1	0	7.9	7.9	1	1.58
		2	7.9	15.8	7.9	1	1.58
16-02-2019	Control	1	0	7.9	7.9	1	1.58
		2	7.9	15.8	7.9	1	1.58
	Leachate	1	0	7.3	7.3	1	1.46
		2	7.3	14.6	7.3	1	1.46
24-02-2019	Control	1	0	7.9	7.9	1	158
		2	15.3	23.2	7.9	1	158
	Leachate	1	0	7.1	7.1	1	1.42
		2	7.1	14.2	7.1	1	1.42

Table 2: Oxalic acid production was estimated from fermented broth through titration assay.

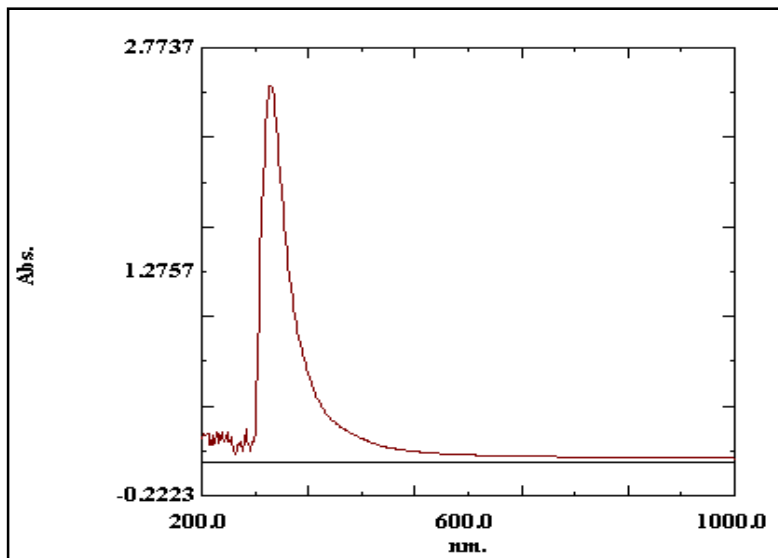
Days	Sample taken	Trial No.	Initial reading	Final reading	Vol. of NaOH consumed	Strength of NaOH	Strength of oxalic acid
10 days	Control	1	0.00	10.00	10.00	0.10	0.200
		2	10.00	20.00	10.00	0.10	0.200
	Broth-1	1	0.00	10.40	10.40	0.10	0.208
		2	11.00	21.40	10.40	0.10	0.208
	Broth-2	1	0.00	10.80	10.80	0.10	0.216
		2	11.00	21.80	10.80	0.10	0.216
20 days	Control	1	13.10	18.50	5.40	0.10	0.109
		2	24.50	30.00	5.50	0.10	0.109
	Broth-1	1	0.00	3.90	3.90	0.10	0.078
		2	3.90	7.80	3.90	0.10	0.078
	Broth-2	1	7.80	10.50	2.70	0.10	0.053
		2	10.50	13.10	2.60	0.10	0.053

zu UV visible-1650 PC spectrophotometer. The spectra of both control and treated broth are displayed below.

The absorption (λ_{max}) of the control was observed in the range of 304 nm (Fig. 5). But in the case of broth two, an additional peak was observed in the range of 524 nm, which confirms the formation of a complex metal with oxalic acid (Fig. 6).

The effect of zinc oxide, zinc hydroxide, and nickel hydroxide on the cell growth in terms of the dry weight of biomass at different concentrations was reported (Table

3, 4 and 5) (Fig. 7, 8 and 9). It can be seen that a high concentration of zinc inhibited growth up to control values. A higher concentration of zinc accumulates in a concentration-dependent manner up to 60 ppm in the medium. *A. niger* was fairly effective in removing zinc oxide, zinc hydroxide, and nickel hydroxide from metal solution at a concentration ranging from 20-100 ppm. The results showed the concentration of heavy metals and nanoparticles remaining in the solution and accumulated by the organism.



Sl. No.	Wave length (nm)	Absorption
1	956.0	0.030
2	304.0	2.524
3	274.0	0.074

Fig. 5: Spectra analysis of control broth.

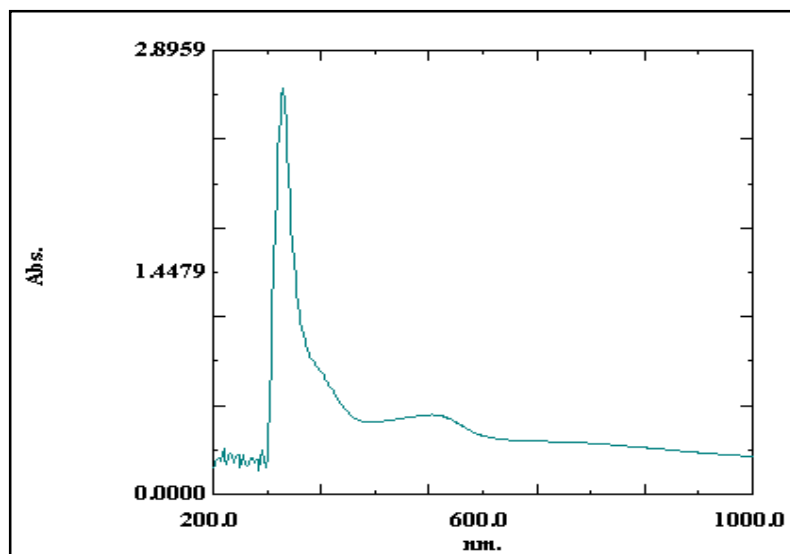


Fig. 6: Spectra analysis of treated broth.

Sl. No.	Wave length (nm)	Absorption
1	524.0	0.518
2	304.0	2.646
3	434.0	0.467

 Table 3: Bioaccumulation of zinc oxide (Heavy metals) by *Aspergillus niger*.

Initial conc. (mg/L)	Graph value	Conc. of metal remaining (mg/L)	conc. of metal accumulation (mg/L)	% of removal	Dry wt. of Biomass (mg)
Control	0	0	0	0	343
20	3.1830	4.085	15.915	75%	315
40	6.5598	7.201	32.799	80%	280
60	6.120	29.35	30.65	50%	259
80	5.9909	50.0455	29.9545	36%	180
100	5.1204	74.398	25.602	25%	180

 Table 4: Bioaccumulation of zinc hydroxide (nanoparticle) by *Aspergillus niger*.

Initial conc. (mg/L)	Graph value	Conc. of zinc hydroxide in metal solution (mg/L)	conc. of zinc hydroxide accumulation (mg/L)	% of removal of zinc	Dry wt. of Biomass (mg)
Control	00	0	00	0	260
20	1.7320	11.34	8.66	40%	175
40	1.5540	32.23	7.70	19.4%	143
60	1.5192	52.404	7.596	12.5%	90
80	1.19161	74.042	5.958	7.3%	75
100	0.5862	97.069	2.931	2.9%	60

DISCUSSION

Water plays an important role in the world economy. The majority (71%) of the Earth's surface is covered by water, but freshwater constitutes a minuscule fraction (3%) of the total. Water fit for human consumption is obtained from freshwater bodies. Approximately, 70% of the freshwater goes to agriculture. This natural resource is becoming scarce

at many places and its unavailability is a major social and economic concern (Khatik et al. 2006, Lohani et al. 2007, Ahmady-Asbchin & Bahrami 2011). Though access to safe drinking water has improved over the last few decades, it is estimated that five million deaths per year are caused due to consumption of polluted drinking water or drought. In many developing countries, 90% of all wastewater still

Table 5: Bioaccumulation of nickel hydroxide (nanoparticles) by *Aspergillus niger*.

Initial conc. (mg/L)	Graph value	Conc. of nickel hydroxide in metal solution (mg/L)	conc. of nickel accumulation (mg/L)	% of removal of nickel	Dry wt. of biomass (mg)
Control	0	0	0	0	290
20	2.2640	8.68	11.32	55%	147
40	1.8903	30.5485	9.4515	22%	90
60	1.5540	52.23	7.77	11.6%	75
80	1.0822	74.589	5.411	6.7%	60
100	0.6264	96.868	3.132	3.1%	60

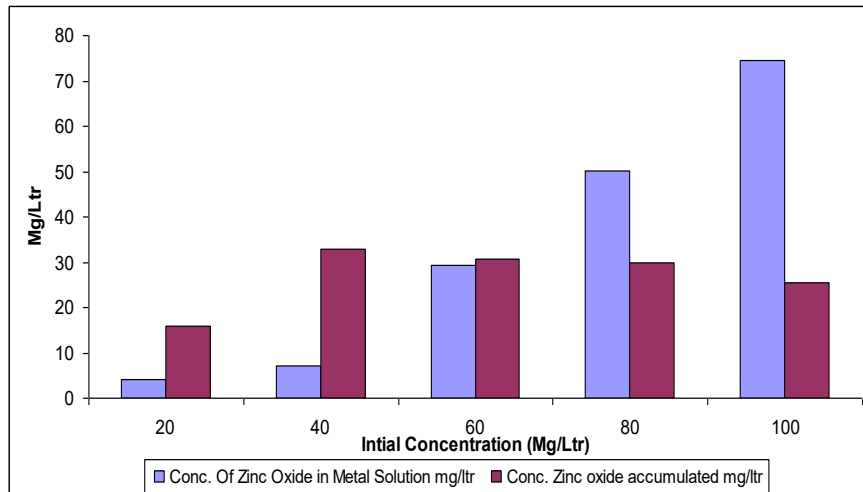


Fig. 7: Graph indicating the residual and accumulated concentration of zinc oxide.

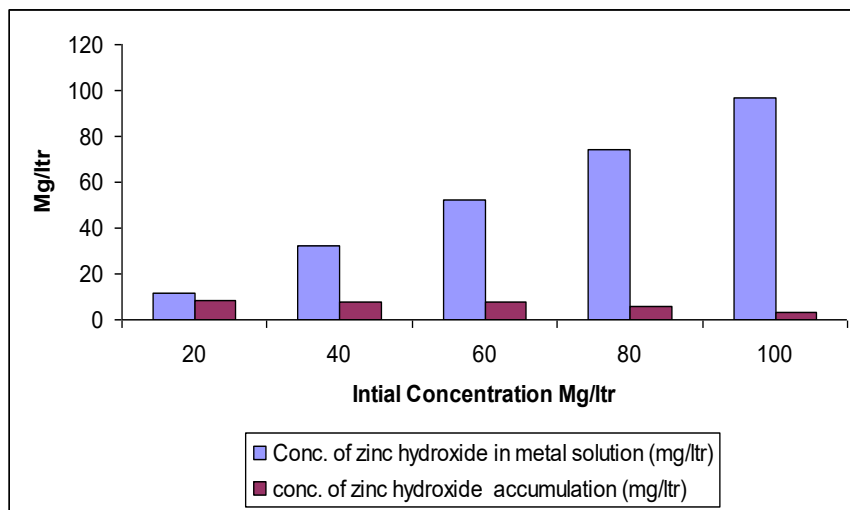


Fig. 8: Graph indicating the residual and accumulated concentration of zinc hydroxide.

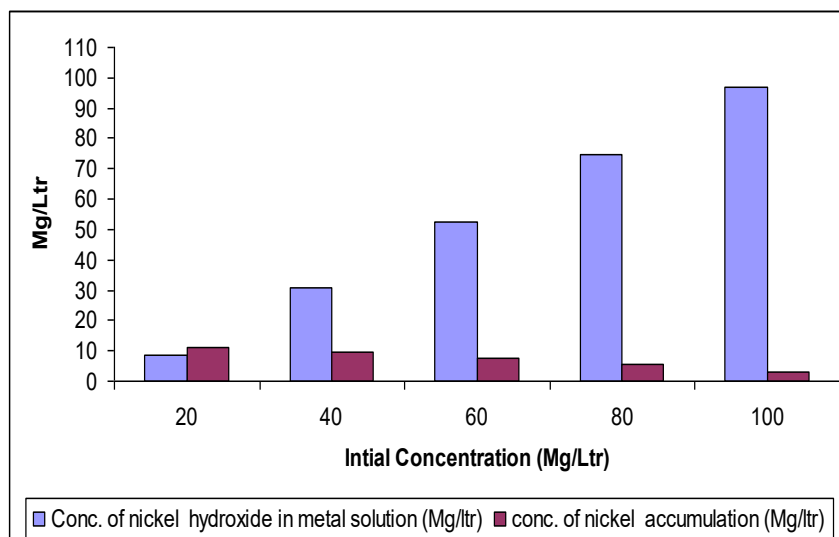


Fig. 9: Graph indicating the residual and accumulated concentration of nickel hydroxide.

goes untreated into the freshwater bodies making it unfit for human consumption, which either leads to scarcity or affects the human population. The concern to protect freshwater bodies for a healthy population is a challenge in recent times. Industrialization to a larger degree is responsible for the contamination of the environment especially water where lakes and rivers are overwhelmed with a large number of toxic substances (Muraleedharan et al. 1991, Murali et al. 2003, Korrapati & Pydi Setty 2012).

Heavy metals are reaching hazardous levels when compared with other toxic substances. Heavy metals are a unique group of naturally occurring compounds. Their continuous release leads to overconsumption and accumulation (Rai et al. 1998, Dhar et al. 2005, Essoka & Umaru 2006, Fawzy et al. 2017). As a result, people around the globe are exposed to adverse consequences of these heavy metals. Many industries (fertilizers, metallurgy, leather, aerospace, photography, mining, electroplating, pesticide, surface finishing, iron and steel, energy and fuel production, electrolysis, metal surface treating, electro-osmosis, and appliance manufacturing) discharge waste containing heavy metals either directly or indirectly into the water resources. Toxic heavy metals, which are of concern are chromium (Cr), lead (Pb), zinc (Zn), arsenic (As), copper (Cu), nickel (Ni), cobalt (Co), cadmium (Cd), and mercury (Hg) (Seide et al. 2000, Vlatka et al. 2001, Siddiquee et al. 2015). As these metals are not biodegradable, they tend to accumulate in living organisms and lead to various diseases and disorders which ultimately threaten human life. They can cause ill health, even when present in the range of parts per billion (ppb). Biosorption has emerged as an attractive option over conventional methods

for the removal of heavy metal ions from effluents discharged from various industries which ultimately reach and pollute freshwater bodies (Samal et al. 2004, Parihar et al. 2007, Ruta et al. 2010).

The bioaccumulation capacity of *A. niger* over a concentration ranging from 20 ppm to 100 ppm was estimated for zinc oxide heavy metal, zinc hydroxide nanoparticle, and nickel hydroxide. *A. niger* was fairly effective in removing zinc oxide, nanoparticles like zinc hydroxide, and nickel hydroxide from the media supplemented with metal solution observed in Figs. 7, 8 and 9.

At low concentrations, such as 20 ppm, there was a nearly complete accumulation of heavy metal (zinc oxide) in the medium. As the concentration of heavy metal increased in the medium, accumulation by the organisms varied. There was a decrease in the percentage of uptake of metal by fungal biomass. There was 80% removal of zinc oxide heavy metal at an initial concentration of 20 ppm to 25% at a final concentration of 100 ppm.

The results were compared with the work done by Asha & Juwarkar (1988). In her work on bioaccumulation of zinc by *Penicillium* sp., the percentage removal of zinc was 70% at a concentration of 100 ppm to 25% at a final concentration of 600 ppm.

Elizabeth & Priyadarshini (2004) had reported that metal up to a concentration of 50 ppm inhibits the growth of organisms. It can be seen that a high concentration of zinc inhibited the growth of the organism than control. Growth of organism was observed on the third day of incubation in control but in test samples, growth of organism was observed

on the seventh day. This reflects that metals had an effect on the growth of the organism.

The effect of nanoparticles on the accumulation and growth of organisms was comparatively low when compared to that of heavy metals. The accumulation of zinc hydroxide drastically reduced from 40% to 2.9% from an initial concentration of 20 ppm to a final concentration of 100 ppm respectively. Likewise, nickel had an effect on growth and accumulation. The percentage removal of nickel hydroxide reduced from 55% at 20 ppm to 3.1% at 100 ppm. This present work was compared with the work conducted by Hussain et al. (2004), who investigated the effect of carbon-60 on two common soil bacteria *E. coli* and *B. subtilis*. At a concentration of 2.5 mg.L⁻¹ of carbon-60, inhibition of the bacterial growth was observed. Reduction in the dry weight of biomass was observed as the concentration of metal i.e. nanoparticles increased in the medium.

Microorganisms capable of tolerating unfavorable conditions evolved their use as biosorbents in the removal of metal ions from wastewaters. They include bacteria, yeast, algae, and fungi. Experiments focused on the use of dead and or living microorganisms offer options for the type of remediation to perform. However, the use of dead microbial biomass for the binding of metal ions has been preferred over living biomass because of the absence of the requirement of nutrients and monitoring BOD and COD in effluents. Hence, the use of dead biomass is economical. These biosorbents can effectively sequester metal ions in the solution and decrease the concentration from the ppm to ppb level efficiently; therefore, they are considered ideal candidates for the treatment of complex wastewaters with high volume and low concentration of metal ions. A large quantity of materials of microbial origin has been investigated as biosorbents for the removal of metal ions extensively. Reports do not include the use biomass of any pathogens for water treatment (Rezza et al. 1997, Barros et al. 2003, Dhar et al. 2005, Chatterjee et al. 2006).

Fungi are also considered economic and eco-friendly biosorbents because of their characteristic features, that is, easy to grow, high yield of biomass, and ease of modification (chemically and genetically). The cell wall of fungi shows excellent binding properties because of distinguishing features like chitin, lipids, polyphosphates, and proteins among different species of fungi. The cell wall of fungi is rich in polysaccharides and glycoprotein which contain various metal-binding groups like amines, phosphates, carboxyls, and hydroxyls. Fungal organisms are used in a wide variety of fermentation processes. Hence, they can be easily produced at the industrial level for the biosorption of metal ions from a large volume of contaminated water resources. Besides, the

biomass can be easily and cheaply obtained from inexpensive growth media or even as by-products from many fermentation industries. Further, fungi are less sensitive to variations in nutrients and other process parameters like pH, temperature, and aeration. Because of their filamentous nature, they are easy to separate by means of simple techniques like filtration (Khasim Beebi et al. 1999, Ghaed et al. 2001, Rajendran et al. 2003, Segaran et al. 2020).

In the present study, we have isolated the *A. niger* from industrial effluent. This strain is more efficient for metal absorption from industrial water. This strain is widely used for the bioleaching process. Production of oxalic acid was using malt extract broth by submerged fermentation. The processed sample was found to be containing heavy metals like zinc, copper, cadmium, lead, cobalt, and nickel.

The mechanism of biosorption is a complex process that involves the binding of sorbate onto the biosorbent. Many natural materials can be used as biosorbents which involve the seventy-four biosorption binding of metal ions by physical (electrostatic interaction or van der Waals forces) or chemical (displacement of either bound metal cations (ion exchange) or protons) binding, chelation, reduction, precipitation, and complexation. Biosorbents contain chemical/functional groups like amine, amide, imidazole, thioether, sulfonate, carbonyl, sulfhydryl, carboxyl, phosphodiester, phenolic, imine, and phosphate groups that can attract and sequester metal ions (Ramteka 2000, Sarkar & Gupta 2003, Srinath et al. 2003).

A. niger is a heterotrophic microorganism that is widely applied in current bioleaching technology and has been extensively researched by local and foreign scholars. To date, bioleaching technology research is mainly related to metal removal in urban fly ash, waste catalyze, and minerals, and is mainly engaged in the screening and optimization of bioleaching process conditions. However, research on the leaching of heavy metals in urban sludge by *A. niger*, as well as on agricultural adaptability and environmental risks after sludge leaching, are lacking. The toxicity of heavy metals not only depends on concentration but also revolves around bioavailability.

CONCLUSION

It can be concluded that both titrimetric data and UV spectral data confirm the decrease in the acidity and complex formation respectively, which in turn confirms the bioleaching process of the metals extracted from the industrial effluent. Decrease in the individual metal concentrations will be carried out and the use of the bioleaching process in the removal of metal from the effluents has to be evaluated. The effect of

nanoparticles on the accumulation and growth of organisms was comparatively low when compared to that of heavy metals. The accumulation of zinc hydroxide drastically reduced from 40% to 2.9% from an initial concentration of 20 ppm to a final concentration of 100 ppm respectively. Likewise, nickel had an effect on growth and accumulation. The percentage removal of nickel hydroxide reduced from 55% at 20 ppm to 3.1% at 100 ppm. Finally, these results suggest the potential applicability of *A. niger* for the remediation of heavy metals from polluted industrial effluent.

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