Bioleaching of nickel and cadmium metals from spent batteries using *Aspergillus flavus*

Divya K. and Abraham Jayanthi*
Microbial Biotechnology Laboratory, School of Biosciences and Technology, VIT University, Vellore, Tamil Nadu, INDIA
*jayanthi.abraham@gmail.com

Abstract

This study focuses on the recovery of heavy metals from e-waste using a fungus isolated from a soil sample collected from a garbage site. Molecular identification of the isolate revealed the organism to be Aspergillus flavus and was designated as AJC5. The isolate was subjected to heavy metal uptake through two mechanisms: bioaccumulation and biosorption. Atomic absorption spectroscopy (AAS) was primarily used to analyse the uptake of heavy metal ions and was further confirmed by Scanning electron microscopy (SEM).

Adsorption isotherm assessment was conducted for the biosorption activity of strain AJC5 which revealed that the process followed the Langmuir and the Freundlich modes of isotherm. The adsorbed heavy metals were comprehensively desorbed from the metal-loaded sorbent using HCl and NaOH. The activity of A. flavus strain AJC5 in bioleaching of metals from spent batteries was studied and analysed using AAS, X-ray diffraction, SEM and FTIR.

Keywords: Bioaccumulation, Desorption, Aspergillus, Isotherm studies, Electronic waste.

Introduction

The rapid advancement of technology and the decreasing lifespan of electronic gadgets ultimately increase the volume of electronic waste (E-waste). Heavy metal contamination from unregulated disposal poses a threat to the environment and is gaining national and international attention. According to Husaini et al[20], about half of Thailand's e-waste is disposed of in landfills and these garbage dumps are called as "poisonous time bombs". One of the major constituents of e-waste is batteries. There are various kinds of batteries, notably among them is Ni-Cd.

Cadmium is commonly used in electronic gadgets like tablets, laptops, cell phones etc. Cadmium exposure affects vital organs, the skeletal septum and is proven to cause cancer in humans[13]. Nickel is a micronutrient necessary in our diet but it is also one of the most common metals that cause allergies, affecting between 10 and 15 percent of the world's population. Higher concentrations of nickel are proven to be carcinogenic and may affect the kidneys, liver, muscles and brain when inhaled in large amounts[11]. There are numerous benefits to the bioleaching process including minimal usage of energy, less reaction conditions and environmental friendliness. It is also suitable for polluted soils and low-grade tailings from mining. Applying microorganisms for metal extraction processes has led to the rapid development of bioleaching in recent years[28,31,33].

Autotrophic fungi have been utilized to solubilize a variety of metals in bioleaching. Bioleaching is an adaptable, selective and eco-friendly method that uses the biomass and metabolic products of certain microbes[25]. Fungi are more successful at bioleaching alkaline materials because they can thrive at high pH levels[6]. In addition, excreting organic acids that chelate metal ions, can result in leaching metals quickly with a brief lag phase.

Numerous organisms are efficient in metal absorption among which *Aspergillus, Penicillium* and other fungi have been reported to be effective in removing heavy metals from soil[36,50]. Fungal cell walls contain a wide variety of proteins and polysaccharides. Microbial biomass has proven to exhibit bio sorbent properties in the biosorption process by scientists. The biomass of *Aspergillus* sp., both active and heat-killed forms have been used in biosorption processes [12]. In the present study, *Aspergillus flavus* has been used for its capabilities against metal tolerance and biodegradation properties.

Material and Methods

Chemicals used: The chemicals and solvents used for the study were of high purity and analytical grade.

Aqueous solution of heavy metals: Stock solutions of cadmium and nickel were prepared individually with nickel sulphate $NiSO_4$ and cadmium sulphate $CdSO_4$.

Collection of soil samples: The soil samples were collected from heavy metal contaminated sites in Vellore, Tamil Nadu, India (12.9165°N,79.1325° E). The samples were air dried, sieved and used for the isolation of fungi.

Isolation of fungi using the enrichment technique: In potato dextrose agar (PDA) medium, the fungus was isolated using the enrichment culture technique. The treated soil was inoculated with the enrichment medium (which contains 2 g of $NaNO_3$, 0.5 g of KCl, 0.5 g of $MgSO_4.7H_2O$, 0.5 g of glucose, 10 g of $FeCl_3$, 10 mg of $BaCl_2$, 0.2 g and 0.05 g of $CaCl_2$ in 1000 ml) spiked with 1 mg of Cd (II) and Ni (II). The medium was then incubated for seven days at 120 rpm and 28 ± 2 °C. An aliquot of the culture was transferred to new enrichment medium containing Cd (II) and Ni (II) following the incubation time. A pure culture was obtained by spreading 100 µl of the enrichment medium sample onto heavy metal-containing PDA (Potato dextrose agar) plates

and was sub cultured. Fungi were inoculated in Sabouraud's dextrose broth (SDB), Czapek Dox broth (CDB) and minimal (M1) media to compare their mycelial development[1].

Morphology investigations of fungi: Lactophenol cotton blue staining was performed for preliminary analysis of fungal mycelium and was also observed under a Scanning electron microscope. Molecular identifications of heavy metal tolerant fungal strains were done using 18S rRNA sequencing[14].

Molecular identifications: The isolated fungal strain was characterized molecularly by 18S rRNA sequence analysis. The fungal genomic DNA was collected using the AMpure Fungal gDNA mini kit. This kit recovers DNA from fungal cells by breaking the cellulose, cell wall and plasma membrane using noncorrosive reagents. The 18S rRNA gene was amplified by polymerase chain reaction (PCR) with primers 50-CGWCGRAANCCTTGTNACGASTTT TACTN-30 and 50-AWGCTACSTGGTTGATCCT SCCAGN-30. 50 mL PCR reaction mix was taken and combined with 50 mg of sample gDNA, 100 mg of forward primer, 100 mg of reverse primer, 2 mL dNTP mixture (10 mm), 5 mL 103 Taq polymerase buffer, 3 U of Taq polymerase enzyme and PCR grade water.

The PCR results acquired were amplified and sequenced using the ABI3730xl genetic analyser (Amnion Biosciences, Bangalore, India). Sequencing results were submitted to the GenBank National Centre for Biotechnology Information (NCBI). The accession number obtained was PQ002488 and designated as *Aspergillus flavus* (AJC5).

Screening of fungal isolates: The ability of the isolated fungal strain to grow in the presence of specific heavy metals was verified using the gradient plate method. A base layer with PDA was precast at a 30° angle to create the gradient in the plates. Individual additions of 1 mg/l of nickel and cadmium were made on the upper layer of PDA, which was then kept aside to solidify. The fungal isolates were added to a gradient of individual plates once both layers had solidified and the plates were then incubated. The growth of mycelium on the plates was used to assess the strains' resistance to heavy metals.

Bioaccumulation of fungal strains: On minimal media, the tolerance level of fungal strains to heavy metals was evaluated. The concentration of heavy metals used in the bioaccumulation investigation was selected based on the organism's capacity for tolerance. The bioaccumulation assays were conducted in PDB media using metals and one millilitre of spore solution as an inoculant. At regular intervals, the bioaccumulation of heavy metals by fungi was examined. The percentage of bioaccumulation was used to calculate the efficiency of bioaccumulation:

$$x = \frac{ci-cf}{ci} \text{x } 100$$

where x is concentration of heavy metals accumulated by fungal biomass, ci is Initial concentration of heavy metals in 1 mg/l and cf is final concentration of heavy metals in mg/l.

Enzyme activity: To determine whether the isolate secreted ligninolytic enzymes such as laccase, manganese peroxidase and lignin peroxidase, the isolate was inoculated in PDB both with and without heavy metals at a concentration of 1 ppm in separate flasks. Samples were withdrawn from the flasks every 24 hr and centrifuged for 20 mins at 4000 rpm. Additional experiments were conducted using the supernatant.

Laccase enzyme activity was measured using 2, 20-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) as the substrate. Laccase secretion by the strain is indicated by oxidation of ABTS. 100 µl of culture supernatant, 2.8 ml of 0.1 M sodium acetate buffer (pH 4.5) and 0.5 mM ABTS made up the assay's reaction mixture. To measure absorbance at 420 nm in a UV–VIS spectrophotometer (AU-2701, Systronics) against a suitable blank, the reaction mixture was incubated in the dark for 15 mins [27].

Manganese sulphate oxidation was used to confirm manganese peroxidase (MnP) activity. 2.5 ml of 20 mM sodium tartrate buffer (pH 4.5) was added to 1 ml of MnS_O4 (1 mM), along with 1 ml of supernatant and 0.5 ml of H_2O_2 (2 mM). The reaction mixture was then allowed to sit at room temperature for 15 mins. A suitable blank was utilized to compare the absorbance which was measured at 238 mins. The substrate utilized to measure the synthesis of lignin peroxidase (LiP) was veratryl alcohol. 1.25 ml of sodium tartrate buffer (50 mM, pH 2.5), 0.5 ml of supernatant, 0.5 ml of 500 µM H_2O and 0.25 ml of vertary alcohol (2 mM) were combined to create the reaction mixture. After 15 mins of incubation, the absorbance was measured at 310 nm[16]. Units/millilitre (U/ml) is the unit of measuring for enzyme activity and 1 U denotes 1 mmol of the substrate that is oxidized each minute.

Atomic absorption spectroscopy (AAS): The heavy metal concentration in the liquid medium before and after the biosorption experiment process was identified using Atomic absorption spectroscopy (AAS) (model no. Varian spectra A240). The filtrate collected after the experiments was subjected to AAS which was further compared with the heavy metal content in the control sample. To prepare a stock solution of nickel, cadmium and nickel cadmium ion (100 ml), required amount of salts for 100,200 and 300 PPM of nickel sulphate, cadmium sulphate (SRL company). The spent nickel cadmium battery powder was dissolved in distilled water and kept in an orbital shaker for 15 mins at 100 rpm and left undisturbed for 24 hrs for complete dissolution. The stock solution was diluted with deionized distilled water to achieve the desired concentrations [5]. In order to maintain a proper pH, solutions were adjusted with 0.1 N NaOH and 0.1 N HNO_3. The cadmium concentration

was measured using an Atomic absorption spectrophotometer.

Scanning electron microscopy (SEM): The surface morphology of fungal mycelium and biosorbent and changes that occurred during the experimental test, were studied thoroughly using SEM (model: Zeiss Evo 18). The samples were rinsed with 70%,80% and 100% ethanol. Once dried thoroughly, they were coated with gold using a sputter coater to avoid charging of the samples. The specimens were analysed and maintained at 10kV[3] at high vacuum.

Fourier transform infrared spectroscopy (FTIR): The FTIR spectra of fungus mycelium and biosorbent were checked before the experimental investigation to determine the presence of metal ions adhering to the functional group. The alterations in bonding that occurred on the fungal mycelium and biosorbent following metal ion uptake were investigated and compared. Helium-neon laser light was utilized to generate infrared radiation. The recording spectra ranged from 4000 to 500 cm^{-1}. The specimens were prepared using potassium bromide[11]. The FTIR model utilized in the study was of Shimadzu 8400, Japan, using Hyper IR-1.7 software for Windows.

The bioleaching of metals from spent batteries

Preparation of spent batteries: The spent Ni-Cd batteries were collected from an electronics shop near Vellore Institute of Technology. The batteries were manually dismantled. All dismantled materials were collected and safely discarded except for the electrode powder materials. The powder was dried, ground by milling and sieved to obtain a mesh size of less than 200 ml [33]. To determine the metal content, the powder was dissolved using the USEPA method 3050B (USEPA, 1986).

Desorption study: To determine the desorption efficacy of Cd (II), Ni (II) and Ni-Cd by *A. flavus* strain AJC5 strain, acidic and alkaline agents are important factors. Several researchers have reported the application of HCl and NaOH in desorption of metal ions from biosorbent and inorganic adsorbents [14,53].

Bioaccumulation of nickel and cadmium by immobilized fungus: The modified protocol of Shekhar et al[47] was followed to immobilize the cells. The fungal culture exponentially increased and the mycelial cells were aseptically extracted and placed in a one litre container using a spatula and homogenised in a blender. 5 ml of fungal cells were homogenized with 150 ml of distilled water in a 250 ml conical flask and carefully mixed and allowed to settle for 10 mins. 3g of sodium alginate was added to the calcium chloride solution. After which the mixture was added dropwise through a 5 ml syringe into a flask that held 100 ml of sterile 0.554g calcium chloride solution.

This mixture was allowed to settle for one hour to facilitate precipitation and form spherical beads. Following immobilisation, the cells were maintained at 4°C. After being incubated for 72 hr in a conical flask with 50 ml of samples, the known amount of immobilized fungal beads was centrifuged for 20 mins at 6000 rpm. The percentage of each metal was determined using an atomic absorption spectrophotometer.

Results and Discussion

Adsorption isotherm: The adsorption isotherm was used to define the relationship between the fungal absorbent and the heavy metals. The adsorption equations are described by the Langmuir and the Freundlich isotherm models. The Langmuir isotherm equation is given as follows:

$$\frac{Ce}{Qe} = \frac{Ce}{Qm} + \frac{I}{KLQm}$$

where *Qe* represents the equilibrium of metal concentrations uptake by *A. flavus* AJC5, *Ce* is the equilibrium concentration of metal present in the solution, *Qmax* represents the sorption capacity of the biosorbents and *KL* is the Langmuir constant. The Langmuir isotherm model with positive intercepts and slopes confirms the reaction behaviour of heavy metal uptake by the fungus which follows the Langmuir and the Freundlich isotherm models.

$$logQe = logKF\frac{1}{n}\,logCe$$

The equation KF denotes the Freundlich isotherm constant and n is the absorption intensity [2]. The correlation coefficient for nickel and cadmium confirms the model. The Freundlich model can be employed for the prediction of heavy metals on the strain AJC5 as bio sorbent. The obtained values for Kf and n are shown in table 1.

The Langmuir isotherm distribution appears as a straight line between Ce /Qe Vs. Ce in the graph in figure 2. The coefficient for nickel is less common than cadmium. The coefficient for nickel on treated fungal strain AJC5 was found to be 0.9727 (Fig.2a), R^2 value for cadmium is 0.9709 (Fig. 2b) and for treated nickel cadmium battery sample, the obtained value is 0.9489 (Fig. 2c). RL values between 0 and 1 validate the favourable absorption of cadmium adsorption.

The Freundlich model is a numerical equation that explains non-ideal adsorption on rough surfaces and multilayer processes[52]. In the experimental study, the correlation coefficients (R^2) for the adsorption of cadmium, nickel and nickel cadmium were observed. The correlation coefficient (R^2) values were inferred based on the adsorption values. The Langmuir isotherm and the Freundlich isotherm fit well with adsorption when compared with the control values.

The correlation coefficient for nickel ions was found to be 0.9727, for cadmium ions, the R^2 value found was 0.9709 and for Ni-Cd ions, the R^2 value found was 0.9489. The Langmuir graph was plotted against Ce Vs. Ce/Qe and is represented in fig. 1. Similarly, for Freundlich isotherm, the

R^2 value for nickel ion on biosorption of fungal strain was found 0.9547, on cadmium ions, the R^2 value was 0.9814 and on nickel cadmium ions, the R^2 value was 0.9823 and the graphs were plotted against log of Qe Vs. log of Ce. The graph is represented in fig. 2. The high correlation coefficient suggests that the data corresponds well with the Freundlich model.

The percentage of bio adsorption of *Aspergillus* strains of treated and control was derived using the values obtained from atomic absorption spectroscopy. On treated nickel ions the percentage of removal was found to be 90%. For cadmium, the percentage of removal was 95%. When compared with nickel and cadmium ions, the percentage of bio adsorption was found to be high on nickel cadmium ions, reaching a percentage of 97% and the results were compared with the control. There are several reports on *Aspergillus* species claiming that at 50 mg of Cd, 20% of the species failed to grow.

Table 1
Isotherm parameters obtained during biosorption of heavy metals nickel and cadmium on fungal strain *Aspergillus flavus* (AJC5).

S.N.	Heavy metals	Isotherm models					
		Langmuir model			Freundlich model		
		Q max	K_L	R^2	K_F	n	R^2
1	Nickel	0.8565	0.0872	0.9727	2.5677	34.354	0.9547
2	Cadmium	1.5432	0.2452	0.9709	29875	1.4354	0.9814
3	Nickel-cadmium	0.4432	0.0765	0.9489	2.6544	1.4765	0.9823

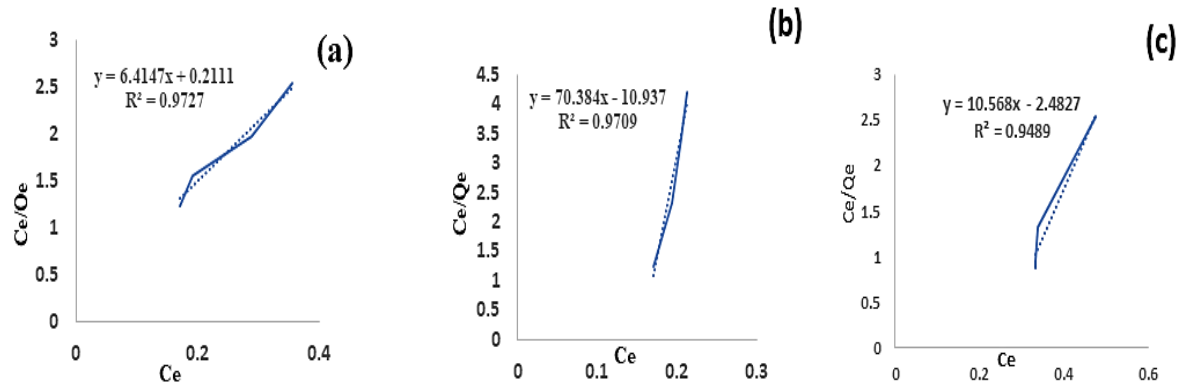

Fig. 1: Langmuir isotherm of fungal strain *A.flavus* (AJC5) (a) Nickel, (b) Cadmium, (c) spent nickel-cadmium battery

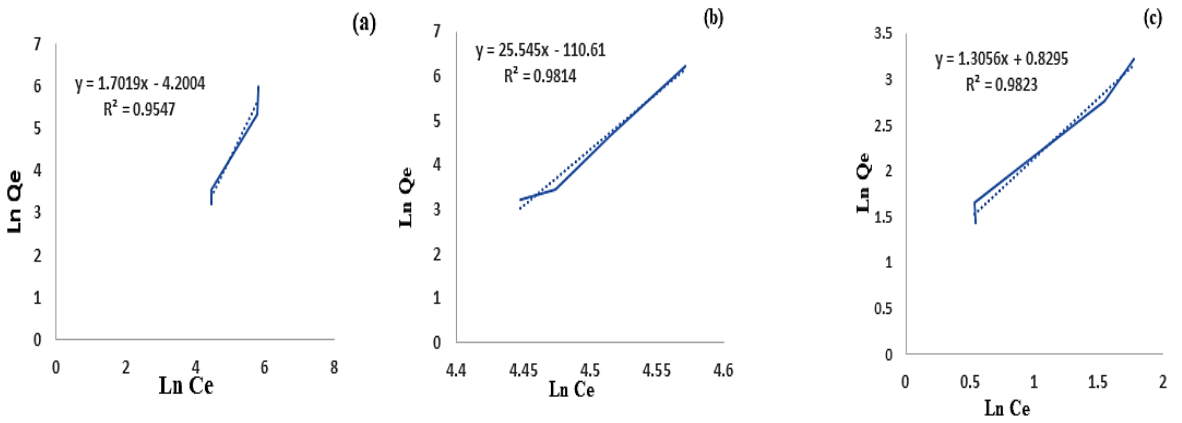

Fig. 2: Freundlich isotherm of fungal strain *A. flavus* (AJC5) (a) Nickel, (b) Cadmium, (c) spent nickel -cadmium battery

At this concentration of Cd, A. *fumigatus* exhibited the highest resistance, with a decrease in growth of 77.7%, while the other *Aspergillus* species exhibited a decline that ranged from 82.2 to 99.4%. 100 mg Cd/100 ml medium killed all isolates except *A. fumigatus* and *P. chrysogenum*. They respond differently to cadmium concentrations as they are morphologically and physiologically different from other fungal strains[30,38,40].

Aspergillus fumigatus showed the maximum tolerance to cadmium (Cd), with only a 77.7% growth at the tested Cd concentration whereas other species such as *A. flavus*, *A. niger* and *A. sydowii* demonstrated significantly greater growth inhibition. Their response to Cd was correlated to the species and strains rather than fungal genera due to their inherent morphological and physiological variations[30,51,54]. The highest Ni (II) removal efficiency was 90% at a biomass dose of 1mg/l and at an initial ion concentration of 52.3mg/L. The initial pH of the solution was set to a medium range. In a comparable investigation, Mothe et al[35] observed that the initial Ni (II) ion concentration varied from 10 to 600 mg/L.

The bioleaching approach of the present study aims to extract metal ions from spent battery powders in aqueous media. The spent battery powders contained Ni (30.92%), Cu (55.62%), Fe (13.47%) and Pb (1.74%), along with trace levels of Zn, Al, Cd, Co and Li. During the bioleaching process with *A. niger* strain AJC5, the pH of the medium decreased from nearly 8 to 3 by the seventh day. The reason for reduced pH may be due to organic acids produced by the fungal strain[42]. Around 96% of absorption had been noticed with strain AJC5. The production of organic acids is a major determinant of bioleaching with fungi. The concentration of heavy metals in the control setup was unaltered.

Growth kinetics experiment: The growth kinetics experiment was carried out using parameters that include the biomass concentration and initial metal concentration. The experiment was carried out using 1mg/l concentration of nickel (Ni) and cadmium (Cd) by using the gradient plate method with and without the presence of the metal. The strains devoid of metals were considered as controls. Joshi[23] reported that the growth of fungus was reduced on the first day, however, the growth increased when the incubation was extended for 5 days and the fungus *A. flavus* (AJC5) exhibited better tolerance against Ni and Cd.

Enzymatic assay: The ligninolytic extracellular enzymes by the strain *Aspergillus flavus* AJC5 were determined by enzyme assay with respective substrates. Manganese peroxidase (MnP) and lignin peroxidase (LnP) and laccase were produced by *A. flavus*. The secretion of manganese peroxidase was lower when treated with nickel ions in the presence of *A. flavus* compared with other substrates. There are earlier reports on several other species of *Aspergillus* producing laccase[36], but there is no information regarding laccase production by *A. flavus*. Extracellular enzymes are not protected by detoxification of metals and they react to metals present in the environment, resulting in the tolerance of the enzyme and metals.

Enzyme production for lignin peroxidase production by *A. flavus* was found to be higher on the 8th day when spiked with nickel ions. With cadmium, manganese peroxidase was found to be higher in all 10 days of exposure. When the strain was inoculated in the conical flask with spent battery powder, the interaction resulted in enzyme production and mutual secretion of laccase and manganese peroxidase on the 8th and 10th day was noticed and the results are represented in fig. 4.

FTIR Analysis: The FTIR spectra of fungal biomass grown in cadmium, nickel and nickel cadmium in PDB medium were obtained in the range of 500–4000 cm^{-1} and the obtained values were compared with the control sample. The infrared analysis shows information about functional groups when the fungus was exposed to nickel and cadmium. Control fungal biomass showed a broad and strong peak at 1636 cm^{-1} and 3293 cm^{-1} corresponding to the amide and amine groups[10].

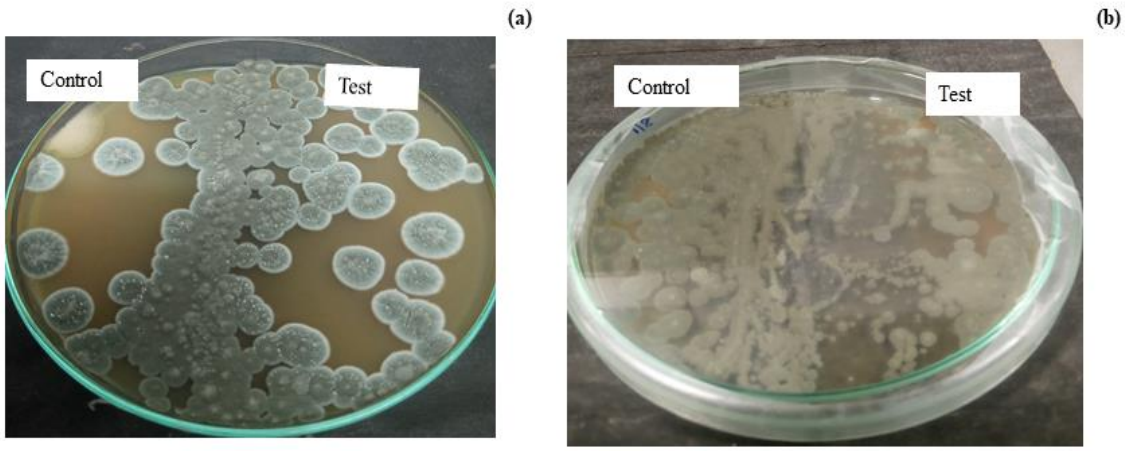

Fig. 3: Gradient plate of (a) 1mg/l of nickel ion, (b) cadmium ions treated with *A. flavus*

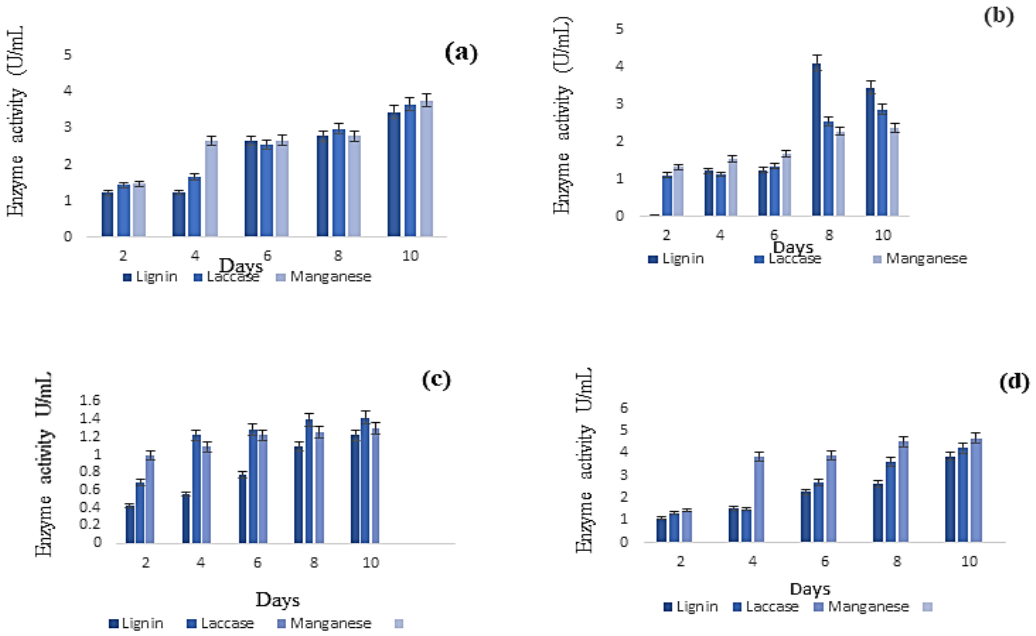

Fig. 4: Graphical representation of enzyme production of (a) control, (b) *A. flavus* treated on nickel ion, (c) enzyme production for cadmium, (d) enzyme production for spent battery nickel- cadmium ions

Fig. 5 shows that when the fungal cells interacted with nickel and cadmium ions, the peaks formed at 3319 cm^{-1}, 2981 cm^{-1}, 1634 cm^{-1} showing stretching of N-H bending [26] and O-H stretching of alcohol group. These peaks correspond to the stretching vibration of C=O in the carboxylic group, N–H and C–N stretching of the amide (III) and stretching of carboxylate anions (–COO–) respectively, in which N–H and C–N have one nitrogen atom that contains one unique pair. A sharp peak was found at 3368 cm^{-1} when the strain AJC5 interacted with the spent nickel cadmium battery.

The peak at 1635 cm^{-1} wavenumbers was the only significant variation in the spectrum. The infrared (IR) analysis of bio sorbents provided valuable insights, particularly in the 1650-1620 cm^{-1} range. This specific band highlights the presence of the amide I bond, which is a hallmark of the amide linkages in poly-N-acetyl glucosamine (chitin) and includes the protein peptide bonds found in biomass. These observations reveal the significant interaction between double bond stretching vibrations, primarily from carbonyl (C=O) groups and hydrogen bonding[24]. The findings show that the functional groups on biomass that were most affected by pH variations, were -OH and -NH and that heavy metal ion uptake took place through these groups.

Scanning Electron Microscope: Scanning electron microscopy was used to examine the surface morphology of the fungus both before and after the adsorption. The samples were cleaned with ethanol at varying concentrations and were air dried. A gold palladium sputter was applied on the samples to prevent the samples from charging before analysis (Ultra 55 model, Zeiss, EVO 18, Germany). SEM analysis was performed before and after 1mg /l of nickel and cadmium treated *A.flavus* biomass to visualize changes in morphological characteristics. Fig. 6(a) shows the image *A.flavus* with the spores of smooth surface. Fig. 6(b) shows a thin and tight layer of nickel which was produced as a result of the nickel electrode position on the carbon fabric.

Fig. 6(c) shows a complete change in *A,flavus* morphology due to its contact with Cd(II). The findings of the present study support the direct, efficient adsorption of heavy metal ions on the bio sorbent *A. flavus.* The percentage weight of phosphorus increased from 0.77% (control) to 1% (1 mg/L treated biomass), which might be indicative of cadmium bio-precipitation as cadmium phosphate bio precipitate. Leusch et al[28] reported sulphur and cadmium spectrum, which illustrated the increase in Cd precipitation by sulphate-reducing organism.

Surface morphology was carefully examined using SEM, as seen in fig. 6. The powder with a smooth surface turned rough with irregular and uneven structures on the pores [30]. From the breakdown of the metals present, the SEM data suggests that the fungal strain is active on the battery powders. Initially, C, Cu, Ni, Li, Zn and Al were detected in the battery powder by XRD analysis. Following treatment with strain AJC5, the residues showed trace amounts of Cu, Zn, Li and Al in addition to the presence of carbon. The XRD pattern also verified the total elimination of Ni.

Bioleaching of metal ions from spent batteries using *Aspergillus flavus* AJC5: Metal ions from spent battery powders were absorbed by an aqueous media using the bioleaching technique. As a reliable, lasting electrochemical system, nickel-cadmium (Ni-Cd) alkaline batteries

consumer demand has grows significantly due to their use in various applications. These are rechargeable secondary storage batteries with an alkaline electrolyte like potassium hydroxide and two electrodes made of nickel oxyhydroxide as the cathode and metallic cadmium as the anode. There are reports of the cathode material from spent Ni–Cd batteries being dissolved efficiently in sulfuric acid[4,31,37]. Nickel is found along with iron, cobalt and cadmium in the leached solutions. There have been numerous attempts to use solvent extraction to remove metals from these solutions in a selective manner[39,44,49]. 47% Ni and 48% Cd make up the cathode of Ni-Cd batteries[8]. The spent nickel cadmium batteries were inoculated with *A. flavus* and kept for incubation for 7 days and the samples were analysed in absorption spectra using AAS. The results are compared and tabulated in table 2.

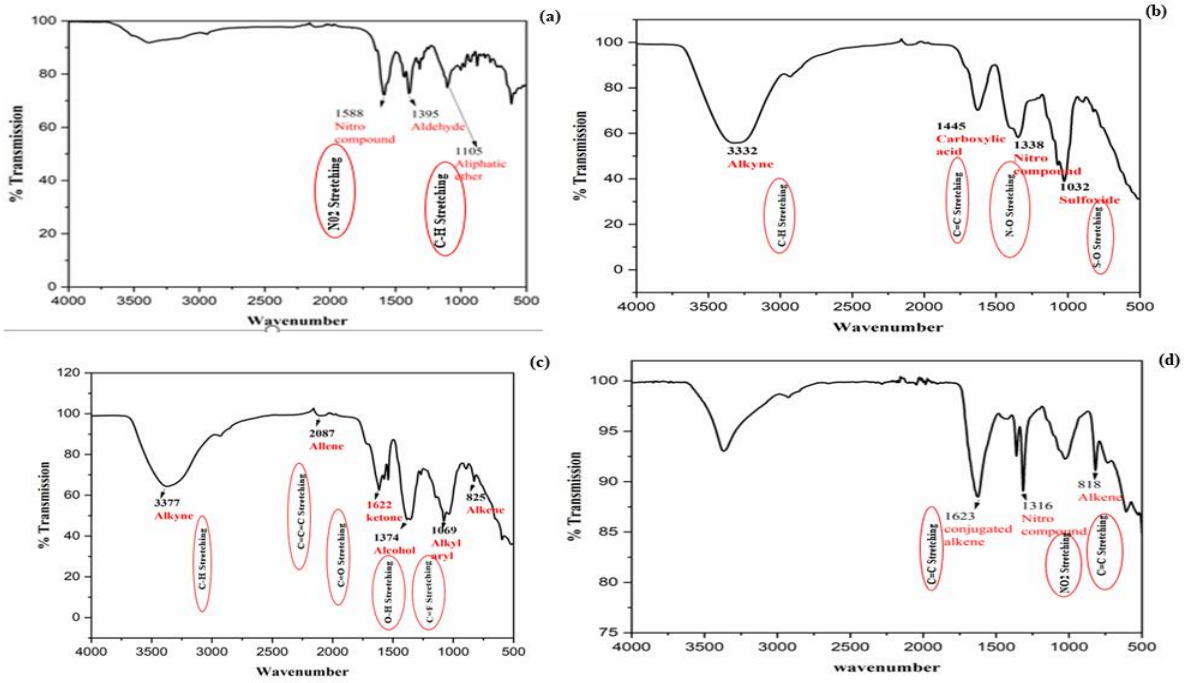

Fig. 5: FTIR spectrum of (a) control, (b) treated nickel, (c) cadmium treated (d) spent nickel cadmium battery treated with *A. flavus*

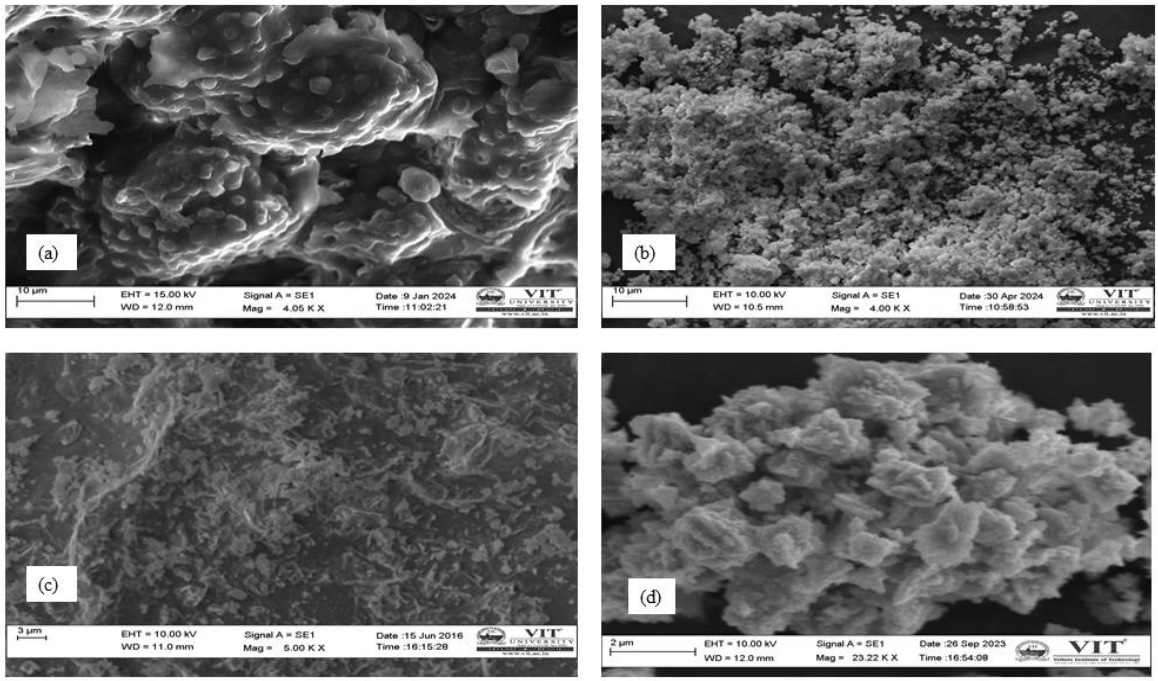

Fig. 6: SEM image of (a) *A. flavus* (control), (b) nickel treated, (c) cadmium treated with *A. flavus,* (d) Spent battery (nickel- cadmium) treated with *A. flavus.*

Table 2
Percentage desorption and quantity of heavy metals on fungal strains AJC5 for nickel, cadmium and spent battery.

S.N.	Bio absorbent	Heavy metals	0.1N HCl		0.1N Na $(OH)_2$		Removal of Heavy metals	
			Desorption percentage	Duration (Min)	Desorption percentage	Duration (Min)	HCL (mg/L)	NaOH (mg/L)
1		Nickel	84%	120	89 %	60	154	132
2		Cadmium	95 %	80	91 %	80	277	243.8
3	*A. flavus* AJC5	Spent battery Nickel-cadmium	93%	120	90%	120	387	456

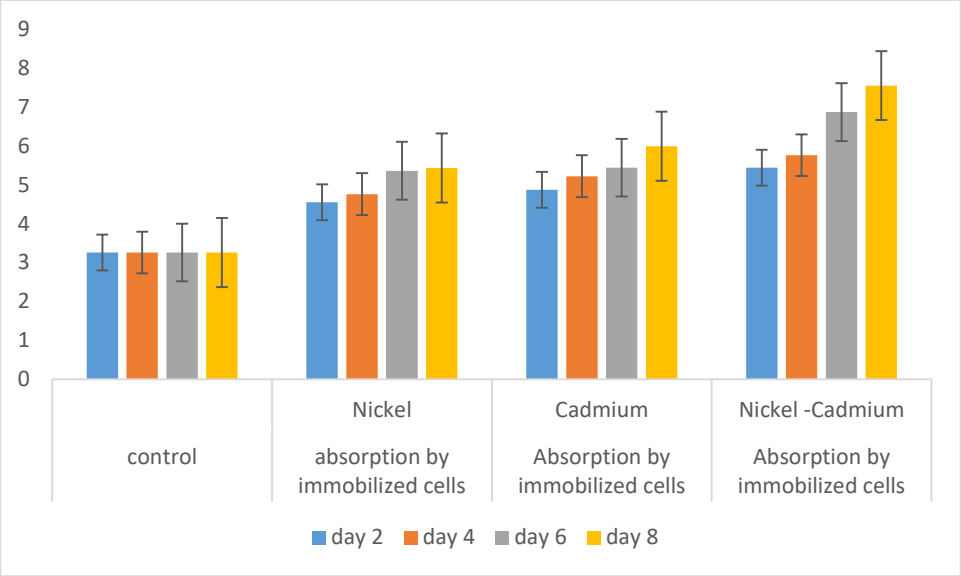

Fig. 7: Bioaccumulation of metal ions using immobilized beads of the strain AJC5

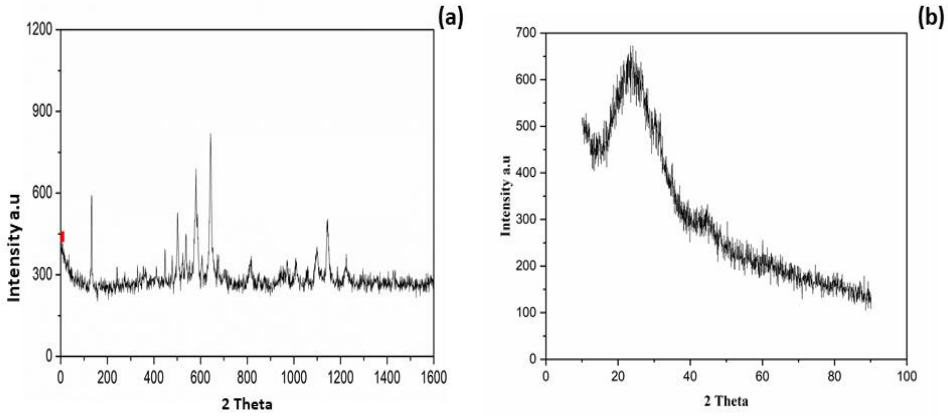

Fig. 8: X-Ray diffraction of (a) Spent nickel -cadmium powder; (b) treated nickel -cadmium powder with *A. flavus* strain.

Desorption and recovery study: The development of heavy metal treatment systems employing *A. flavus* strain as a biosorbent of the spent battery depends on the assessment of the desorption effectiveness of Cd (II) and Ni (II) using acidic and alkaline agents. Table 2 lists the results of the desorption of heavy metals using 0.1 N HCl and 0.1 N NaOH. According to several studies, HCl can be used to desorb metal ions from inorganic adsorbents and biosorbent[15,53]. Compared to HCl, NaOH exhibited a higher desorption for Cd (II) and Ni (II). The high pH of NaOH may have caused the metal ions to become weakly bound to the bio sorbent, which was readily removed[48].

HCl was found to have the highest desorption of Cd (II). Due to its high proton level and acidic pH, HCl substitutes the metal ions on the biosorbent surface by removing them [24]. When adsorption occurs by complexation or ion exchange, an acidic eluent produces a higher desorption rate. Thereby,

the high HCl desorption efficiency during Cd (II) loaded biosorbent regeneration indicated that both ion exchange and complexation were a part of the adsorption process.

To recover the heavy metals, hydrochloric acid and sodium hydroxide solutions were subsequently prepared to produce crude heavy metals. The concentrated heavy metals were collected by HCl evaporation. To evaporate the HCl, the sample was boiled to 108.6°C. After the evaporation of HCl, the salts that were left behind in the beaker contained heavy metals and AAS was used to confirm it. In the case of NaOH, the dialysis process was applied. A dialysis membrane with a pore size of 1.0µm was used for the experiment. After a 24-hour dialysis process to remove sodium hydroxide from the solution, the obtained crude heavy metals were analysed by AAS [21].

Bioaccumulation of nickel and cadmium by immobilized fungus: Immobilized fungal cell of *Aspergillus flavus* was able to absorb Cd (98%) and Ni (83%) respectively. The adsorption capabilities of the immobilized fungal cells were higher than those of the dead cells.[22] The immobilized fungal cells had comparable adsorption capacities for Cu, Cd and Pb. The explanation for this, according to Joshi[23], is that dead fungal cells are made up of tiny particles with low density, weak mechanical properties and minimal stiffness. Therefore, biomass must be immobilized before being tested for biosorption. Immobilized cells offer various benefits such as increased biomass loading, increased reusability and less clogging in continuous flow systems[15]. Adsorption of heavy metals onto calcium alginate beads is dependent on the cell density.

The results showed an increase in bioaccumulation of metal ions with prolonged duration. However, it was confirmed that the bioaccumulation capacity of combined nickel - cadmium ions was higher than the individual accumulation of nickel and cadmium. This may be due to the ion exchange mechanism and a change in pH level.

X-ray diffraction: X-ray examination was used to determine the electrode powder's chemical makeup from the Ni-Cd expended batteries. Cadmium and cadmium hydroxide, along with a trace amount of nickel, make up the anode hydroxide (Figure 7). Nickel hydroxide and nickel oxyhydroxide cover the cathode. Both samples had traces of cobalt. It appears that the anode has a low concentration of Ni and a high concentration of Cd. The AAS analysis also supported this finding. According to the findings of the AAS analysis, the battery's Ni content was 47% for the cathode and 22% for the anode. The cathode and anode had a cadmium concentration of 12.3% and 45% respectively.

Conclusion

An environmentally friendly and effective adsorption of heavy metals like (Ni, Cd) removal has been evaluated with *Aspergillus flavus*. The peak adsorption of Cd (II) and Ni (II) was found at pH 6.0–7.0, with the removal of Cd (II), Ni (II). Metal ion traces are analysed on the surface in SEM-EDX, revealing the first adsorption stage of accumulation. Additionally, a potential bioaccumulation process was confirmed by the FTIR measurement since multiple cycles of adsorption–desorption can be achieved using the strain AJC5 biosorbent, according to its desorption capacity.

The study has explored the efficiency of the *A. flavus* strain AJC5 in the removal of e-waste, which has been confirmed by the bioleaching of battery powders. The study concludes that strain AJC5 is an effective and suitable applicant for the removal of heavy metals from e-waste.

Acknowledgment

The authors would like to express their gratitude to the management of Vellore Institute of Technology, Vellore.

References

1. Ahmad S. and Yadava J.N.S., Infectious mercury resistance and co-transfer R-plasmid among *E. coli* strains, *Indian J Exp Biol*., **26**, 601 **(1988)**

2. Ajenifuja E., Ajao J.A. and Ajayi E.O., Equilibrium adsorption isotherm studies of Cu (II) and Co (II) in high concentration aqueous solutions on Ag–TiO_2-modified kaolinite ceramic adsorbents, *Appl Water Sci*., **7(5)**, 2279–2286 **(2017)**

3. Baldrian P., Interactions of heavy metals with white-rot fungi, *Enzyme Microb Technol*, **32(1)**, 78–91 **(2003)**

4. Bartolozzi M., Braccini G., Bonvini S. and Marconi P.F., Hydrometallurgical recovery process for nickel–cadmium spent batteries, *J. Power Sources*, **55**, 247–250 **(1995)**

5. Budavari S., O'Neil M.J. and Smith A., The Merck index, An Encyclopaedia of Chemical, Drugs and Biologicals, Merck & Co., Inc, Rahway, N.J., U.S.A. **(1989)**

6. Burgstaller W. and Schinner F., Leaching of metals with fungi, *J. Biotechnol*., **27**, 91–116 **(1993)**

7. Burton J.L., Smith E.A., Peterson S.M. and Brown M.T., Degradation of polycyclic aromatic hydrocarbons by *Phanerochaete chrysosporium*, *J. Environ. Chem. Emg*, **7(5)**, 4262–4271 **(2019)**

8. Çeribasi I.H. and Yetis U., Biosorption of Ni (II) and Pb (II) by *Phanerochaete chrysosporium* from a binary metal system–kinetics, *Water SA*, **27(1)**, 15–20 **(2001)**

9. Cerruti C., Curutchet G. and Donati E., Bio-Dissolution of spent nickel–cadmium batteries using *Thiobacillus ferrooxidans*, *J. Biotechnol*., **62(3)**, 209–19 **(1998)**

10. Chatterjee Ankita and Abraham Jayanthi, Desorption of heavy metals from metal loaded sorbents and e-wastes: A review, *Biotechnol Lett*, **41**, 319-333 **(2019)**

11. Chakraborty P. and Abraham J., Comparative study on degradation of norfloxacin and ciprofloxacin by Ganoderma lucidum JAPC1, *Korean Journal of Chemical Engineering*, **34**, 1122–1128 **(2017)**

12. Chhikara S., Hooda A., Rana L. and Dhankhar R., Chromium (VI) biosorption by immobilized *Aspergillus niger* in continuous flow system with special reference to FTIR analysis, *J. Environ. Biol.*, **31**, 561–566 **(2010)**

13. Ciesielczyk F., Bartczak P. and Jesionowski T., Removal of cadmium (II) and lead (II) ions from model aqueous solutions using sol–gel-derived inorganic oxide adsorbent, *Adsorption*, **22(4–6)**, 445–458 **(2016)**

14. Deng S. and Ting Y., Polyethyleneimine-modified fungal biomass as a high-capacity biosorbent for Cr (VI) anions: sorption capacity and uptake mechanisms, *Environ Sci Technol*., **39(21)**, 8490–8496 **(2005)**

15. Dudek-Adamska D., Lech T., Konopka T. and Koscielniak P., Nickel content in human internal organs, *Biol. Trace Elem. Res*, **199(6)**, 2138-2144 **(2021)**

16. Gadd G.M., Fungi and yeast metal accumulation, in microbiol mineral recovery, edited by Ehrlich H.L. and Brierley C.L., Mc. Graw-Hill Book Co., New York, 249 **(1990)**

17. Genchi G., Sinicropi M.S., Lauria G., Carocci A. and Catalano A., The effects of cadmium toxicity, *International Journal of Environmental Research and Public Health*, **17(11)**, 1-24 **(2020)**

18. George D. and Mallery P., SPSS for Windows Step by Step: A Simple Guide and Reference, 18.0 Update, 11th edition, Prentice Hall: Upper Saddle River, NJ **(2010)**

19. Holan Z.R. and Volesky B., Biosorption of lead and nickel by biomass of marine algae, *Biotechnol. Bioeng*., **43**, 1001-1009 **(1994)**

20. Husaini A., Fisol F.A., Yun L.C., Hussain M.H. and Roslan H.A., Lignocellulolytic enzymes produced by tropical white rot fungi during bio pulping of Acacia magnum wood chips, *J Biochem Technol*., **3**, 245–250 **(2011)**

21. Imran B., Khan S.J., Qazi I.A. and Arshad M., Removal and recovery of sodium hydroxide (NaOH) from industrial wastewater by two-stage diffusion dialysis (DD) and electrodialysis (ED) processes, *Desalination and Water Treatment*, **57(17)**, 1548-1554 **(2016)**

22. Johncy Rani M., Hemambika B., Hemapriya J. and Rajesh K.V., Comparative assessment of heavy metal removal by immobilized and dead bacterial cells: A biosorption approach, *J. Environ. Sci. Technol*., **4(2)**, 77-83 **(2010)**

23. Joshi N.C., Heavy metals, conventional methods for heavy metal removal, biosorption and the development of low-cost adsorbent, *Eur J Pharm Med Res*., **4**, 388–393 **(2017)**

24. Khan S.K. and Alam M.S., Role of Ligninolytic enzymes in fungal bioremediation, *Environ. Technol. Rev*., **6(1)**, 1–14 **(2018)**

25. Khandelwal Vishnu Kumar, Application of silver nanoparticles in the degradation of Methylene Blue dye, *Res. J. Chem. Environ.*, **29(1)**, 39-45 **(2025)**

26. Kunacheva C., Juanga J.P. and Visvanathan C., Electrical and electronic waste inventory and management strategies in Bangkok, Thailand, *International Journal of Environment and Waste Management*, **3(1-2)**, 107-119 **(2009)**

27. Lee J.C. and Pandey B.D., Bio-processing of solid wastes and secondary resources for metal extraction – a review, *Waste Management*, **32**, 3–18 **(2012)**

28. Leusch A., Holan Z.R. and Volesky B.J., Biosorption of heavy metals in water supplies production of oil industry, *J. Chemical. Technology and Biotechnology*, **62**, 279-288 **(1995)**

29. Li H., Liu T., Li Z. and Demg L., Low-cost supports used to immobilize fungi and reliable technique f or rem oval hexavalent chromium in wastewater, *Bioresource Technol*ogy, **99**, 2234-2241 **(2008)**

30. Lopez-Errasquin and Vasquez C., Tolerance and Uptake of Heavy Metals by Trichoderma atroviride Isolated from Sludge, *Chemosphere*, **50(1)**, 137-143 **(2003)**

31. Lundgren D.G., Valcova-Valchanova M. and Reed R., Chemical reactions important in bioleaching and bioaccumulations, In Ehrlich H.L. and Holmes D.S., eds., Biotechnology for the Mining, Metal-refining and Fossil Fuel Processing Industries, John Wiley & Sons, New York, 7–22 **(1986)**

32. Majumdar S.S., Das S.K., Saha T., Panda G.C., Bandyopadhyoy T. and Guha A.K., Adsorption behavior of copper ions on *Mucor rouxii* biomass through microscopic and FTIR analysis, *Colloids and Surfaces B: Biointerfaces*, **63(1)**, 138–145 **(2008)**

33. Mishra D. et al, Bioleaching of metals from spent lithium ion secondary batteries using *Acidithiobacillus ferrooxidans*, *Waste Management*, **28(2)**, 333-338 **(2008)**

34. More S.S., Ps R. and Malini S., Isolation, purification and characterization of fungal laccase from Pleurotus sp., *Enzyme Res*., **2011**, 1- 7 **(2011)**

35. Mothe G.K., Pakshirajan K. and Das G., Heavy metal removal from multicomponent system by sulfate reducing bacteria: mechanism and cell surface characterization, *The Journal of Hazardous Materials*, **324A**, 62–70 **(2015)**

36. Mulligan C. and Galvez-Cloutier R., Bioremediation of metal contamination, *Environmental Monitoring Assessment*, **84**, 45-6 **(2003)**

37. Ndlovu S., Biohydrometallurgy for sustainable development in the African minerals industry, *Hydrometallurgy*, **91**, 20–27 **(2008)**

38. Niu Z., Zou Y., Xin B., Chen S., Liu C. and Li Y., Process controls for improving bioleaching performance of both Li and Co from spent lithium ion batteries at high pulp density and its thermodynamics and kinetics exploration, *Chemosphere*, **104**, 92–98 **(2014)**

39. Nogueira C.A. and Margarido F., Leaching behaviour of electrode materials of spent nickel–cadmium batteries in sulphuric acid media, *Hydrometallurgy*, **72**, 111–118 **(2004)**

40. Olson G., Brierley J. and Brierley C., Bioleaching review. Part B: progress in bioleaching: applications of microbial processes by

the minerals industries, *Appl. Microbiol. Biotechnol*, **63**, 249–257 **(2003)**

41. Rani M.J., Hemambika B., Hemapriya J. and Kannan V.R., Comparative assessment of heavy metal removal byimmobilized and dead bacterial cells a biosorption approach, *J. Environ. Sci. Technol*., **4**, 77 **(2010)**

42. Rashid M. et al, Organic acids production and phosphate solubilization by phosphate solubilizing microorganisms (PSM) under *in vitro* conditions, *Pak J. Biol Sci*., **7(2)**, 187–196 **(2004)**

43. Rawlings D., Dew D. and Plessis C., Biomineralization of metal containing ores and concentrates: review, *Trends Biotechnol*, **1**, 38–44 **(2003)**

44. Reddy B.R. and Priya D.N., Chloride leaching and solvent extraction of cadmium, cobalt and nickel from spent nickel-cadmium, batteries using Cyanex 923 and 272, *Journal of Power Sources*, **161**, 1428–1434 **(2006)**

45. Salgado-Delgado R., Ángeles-Castellanos M., Buijs M. and Escobar C., Internal desynchronization in a model of night-work by forced activity in rats, *Neuroscience*, **154(3)**, 922-931 **(2008)**

46. Selatnia A. et al, Biosorption of Cd^{2+} from Aqueous Solution by a NaOH-treated Bacterial Dead *Streptomyces rimosus* Biomass, *Hydrometallurgy*, **75**, 11 – 24 **(2004)**

47. Shekher R., Sehgal S., Kamthania M. and Kumar A., Laccase: microbial sources, production, purification and potential biotechnological applications, *Enzyme Res*., **2011**, 217861 **(2011)**

48. Shinde V.R. et al, Hydrophobic and textured ZnO films deposited by chemical bath deposition: annealing effect, *Applied Surface Science*, **245**, 407–413 **(2005)**

49. Singh N., Adsorption–desorption studies of herbicides on coal fly ash, *Journal of Hazardous Materials*, **168**, 233–237 **(2009)**

50. Skowronski J.M. and Osinska M., Studies on conditions for separation of nickel from solutions after leaching spent Ni–Cd batteries, *Przem. Chem*., **88(7)**, 819–822 **(2009a)**

51. Valix M., Usai F. and Malik R., Fungal bio-leaching of low-grade laterite ores, *Mineral Engineering*, **14**, 197-203 **(2001)**

52. Volesky B., Biosorption of Heavy Metals, CRC Press, Bora Ratan, Ann. Arbor, Boston, USA, 83-90 **(1990)**

53. Weber W.J., Physicochemical process for water quality control, Wiley, New York, USA **(1972)**

54. Witek-Krowiak A., Application of beech sawdust for removal of heavy metals from water: biosorption and desorption studies, *European Journal of Wood and Wood Products*, **71(2)**, 227–236 **(2013)**

55. Zafar S., Aqil F. and Ahmad I., Metal tolerance and biosorption potential of filamentous fungi isolated from metal contaminated agricultural soil, *Bioresource Technology*, **98**, 2557-2561 **(2007)**.

(Received 03rd July 2025, accepted 05th August 2025)