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Efficient Copper Adsorption from Aqueous Solution by *Dictyuchus sterile* Pellets

Rana Hadi Hameed Al-Shammari*†, Shaimaa Satae M. Ali and Moayad Salih Hussin***

* Department of Biology, College of Science, Mustansiriyah of University, Baghdad, Iraq
**Environmental Studies and Research Center, University of Babylon, Iraq
†Corresponding author: Rana Hadi Hameed Al-Shammari; dr.rana@uomustansiriyah.edu.iq

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ABSTRACT

A common heavy metal pollutant of water resources, copper (II), can cause serious health problems or even death. Over the past few years, several filamentous fungi strains have been isolated, identified, and tested for their ability to bio-adsorb heavy metals for potential use in the bio-remediation of copper from wastewater. In this study, variables, including the dosage of fungal pellets, temperature, pH, time, initial copper concentration, and agitation rate, were assessed to select the best conditions for the adsorption of copper by *Dictyuchus sterile* pellets. To identify the active groups responsible for metal adsorption, microscopic observations were made using a light microscope and scanning electron microscope. The copper adsorbent was then analyzed before and after adsorption using an atomic adsorption spectrophotometer and Fourier transform infrared spectroscopy. The ideal adsorption conditions were: fungal pellets with a wet weight of 1 $g.L^{-1}$ at a temperature of 25°C, pH 5.5, the initial copper concentration of 100 ppm, and shaking at a speed of about 250 rpm for 72 h to achieve a removal efficiency rate of 95%. Copper adsorbed with the biomass of the fungal pellets was 57 $mg.g^{-1}$. The use of fungal pellets would be a method that can be used to increase the surface area of adsorption and also is thought to be one of the most cost-effective ways to remove trace metals from polluted water.

INTRODUCTION

Clean water is a significant concern because the world's population is growing, and needs are becoming increasingly urgent (Boretti & Rosa 2019). Industrialization and civilization have significantly advanced, undoubtedly improving living standards and comforts for people, but accidentally upsetting the critical environmental balance that nature had created over a millennium (EPA 2002). These human activities and industrial discharges are contaminating the water. Organic, inorganic, and biological particles comprise these pollutants' three main categories. The primary pollutant in water is heavy metals, which are produced as waste in various industries, including textile, pharmaceutical, and leather (Barakat 2011). Heavy metal removal is essential because they are hazardous, carcinogenic compounds that shouldn't be released into the environment directly (Gautam et al. 2014, Burakov et al. 2018). Trace metals are generally eliminated through chemical and physical processes such as adsorption to inorganic materials, electrodialysis, chemical precipitation, crystallization, coagulation, flocculation, reduction, and ion exchange (Gupta et al. 2011, 2012a, 2012b). However, the removal of metals requires a lot of chemicals and energy and produces byproducts.

On the other hand, biological remediation using microorganisms (algae, bacteria, fungi) has been investigated for their capacity for metal adsorption from polluted waters, with fungi beneficial for this aim due to their accessibility, low cost, and capacity to ingest significant amounts of heavy metals (Bayramoğlu & Arica 2008, Dhankhar & Hooda 2011, Maznah et al. 2012, Kumar et al. 2019). Many fungal genera, *Aspergillus, Fusarium*, *Mucor*, *Penicillium,* and *Saccharomyces* biomass, have been considerable attention because of their large capacity for metal removal (Çeribasi & Yetis 2001, Kiran et al. 2005, Parvathi et al. 2007, Bayramoğlu & Arıca 2008). Since a great deal of work has been done in this field. By adhering to their mycelium from the freshwater shrimp cultivation pond, fungi such as *Pythium* sp., *Dictyuchus sterile*, and *Scytalidium lignicola* were shown to accumulate zinc, lead, and cadmium (Duddridge & Wainwright 1980). The adsorption efficiency

ORCID details of the authors:

Rana Hadi Hameed Al-Shammari: https://orcid.org/0000-0001-8195-9368
Shaimaa Satae M. Ali: https://orcid.org/0000-00031588-7882

of trace metals by terrestrial filamentous fungi has been confirmed by researchers (Damodaran et al. 2013, Dixit et al. 2015, Dusengemungu et al. 2020). Consequently, numerous studies have examined the effectiveness of various fungal species in removing heavy metals from wastewater. Recently, fungi have been among the classes of microorganisms that have received the most attention in the biological remediation of contaminants (Shakya et al. 2016). These heterotrophic microbes can biotransform metal contaminants through chemical alteration or metabolic mechanisms. They are a good option for bioremediation of pollution because they can grow on contaminants and establish extended mycelial networks (Harms et al. 2011). The cell wall of water molds (Oomycetes) is composed mainly of glucan and cellulose (4-20%), the amino acid hydroxyproline, which is considered the site of the dominance of metal binding sites, such as the chemical groups acetamido, amide, phosphate, amino, amine, sulfhydryl, carboxyl, and hydroxyl groups (Anbia & Alvand 2012, Bulgariu & Bulgariu 2012). This research aims to develop an efficient method for copper ion uptake from aqueous solutions employing *Dictyuchus* and growth parameter optimization to maximize heavy metal adsorption.

MATERIALS AND METHODS

Isolation and Preparation of Fungal Pellets

The previously described technique was used to isolate *Dictyuchus sterile* isolates (Dick 1969). As in the previous study, the baiting technique was employed for sample isolation where sesame seeds Sesamum indicum L were used as baits (Dick 1969, Al-Rekabi & 1996). In separate sterile polyethylene bags, samples of decaying plant leaves obtained from the Al-Jaish channel were brought to the mycology laboratory. After gently mixing the sample, 1 mL of river water was added to 10 cm Petri dishes containing 10 mL of sterile distilled water and 3 sterile sesame seeds and boiled for 15 min to kill bacteria. After 7 days of incubation, fungal growth appears on the seeds, which have been cleaned with sterile distilled water and purified by utilizing Czapek-Dox broth for liquid cultivation ($NaNO_3$, K_2HO_4, KCl, $MgSO_4$, $FeSO_4$, 10 g glucose), at 25°C and pH 5.5, with a shaking speed of 150 rpm for five days (1×10^4 spores.mL^{-1} in 500 mL of suspension) (Alrubaie & Al-Shammari 2018).

Incubation Conditions Affecting Removal Efficiency and Mass Adsorption

Adsorption Dosage of Fungal Pellets Biomass and Initial Metal Concentration

The adsorbent dose was optimized using (0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 g) while holding all other variables constant.

Initial copper concentration, pH, and agitation speed, copper were absorbed and removed from the environment by fungal pellets. Initial concentration tests of aqueous copper nitrate were conducted with concentrations of 20, 40, 60, 80, and 100 ppm in a volume of 100 ml. To modify the pH, 1 HCL and 1 NaOH were applied. pH readings were measured with a digital pH meter (3, 5, 7, 9, 11). By adding 1g of fungal pellets to 100 mL of 100 ppm copper solutions and agitating the mixture for 24 h at a speed ranging from 50 to 250 rpm at pH 5.5 at 25 °C, it was possible to study the effect of agitation speed variation on equilibrium (Al-Mamoori et al. 2020).

Optimization of Contact Time and Temperature

The pH, temperature, and rpm were optimized using data from previous batches, and the mass in grams of fungal biomass was maintained in 100 mL copper ions solutions while being shaken. Samples were analyzed at predetermined intervals after 15, 30, 45, 60, and 75 h. pH and contact time constant and increasing temperature, we investigated how pH affects adsorption studies (20, 25, 30, 35, 40 °C).

Fourier Transform Infrared Spectroscopy (FTIR) Characterization and SEM Morphological Examination

The Fourier transform infrared spectra of fungal pellets exposed to copper (at initial metal concentrations of each metal 100 $mg.L^{-1}$, exposure time 75 h and those non-exposed to copper were obtained using an FTIR spectrophotometer. The samples were then dried in an oven at 50°C. the FTIR spectra were recorded at 25°C in the range of 400–4000 cm^{-1}. The light microscope was generally used in the morphological identification of fungi under low power objectives (10X, 40X) and SEM (Bruker, Japan). After adsorption, fungal pellets were collected, washed with distilled water five times, and dried at 70°C to examine their morphology. A surface elemental analysis of the fungal pellets was carried out using SEM after they were fixed in 3% glutaraldehyde in distilled water at 4°C for 3 h. (Zhang et al. 1998).

Trace Metals Concentration Measurement and Statistical Analysis

Atomic adsorption spectroscopy (AAS) was used in these investigations to quantify the concentration of copper. The holo cathode device (Analytikjena AG instrumentation) was used to correct and standardize the wavelength. After being removed from the flask by Whatman No. 1 filter paper that had been dried overnight in an oven at 50 °C fungal, the amount of copper absorbed by the fungal pellets was determined using the equation below (Zhang et al. 1998).

$$M = \frac{Ci - Ce}{F} * V$$

M: Mass of adsorbed metal on the weight of fungal biomass adsorbent ($mg.g^{-1}$)

Ce: Residual metal concentration Residue in solution in equilibrium after Adsorption (ppm)

Ci: Initial metal concentration before adsorption (ppm)

V: volume of solution (L)

F: adsorbent mass of fungal pellets (g)

The equation below was used to calculate the effectiveness of removing trace metals (Anbia and Alvand, 2012).

$$RE\ \% = (Ci - Cf) \times 100\ Ci$$

Ci: Initial metal ion concentration ($mg.L^{-1}$) before adding the fungal pellets

Cf: Residual metal ion concentration ($mg.L^{-1}$) after adding the fungal pellets

Statistical Analysis

The significant difference between various values of the incubation conditions was determined statistically using a two-way ANOVA followed by a student t-test. Each factor was attained with three replicates.

RESULTS AND DISCUSSION

Characteristics and Pellets Formation of *Dictyuchus sterile*

Fungus *Dictyuchus sterile* was isolated from contemned water from the Al-Jaish channel in Baghdad and classified depending on morphological features of colonies and

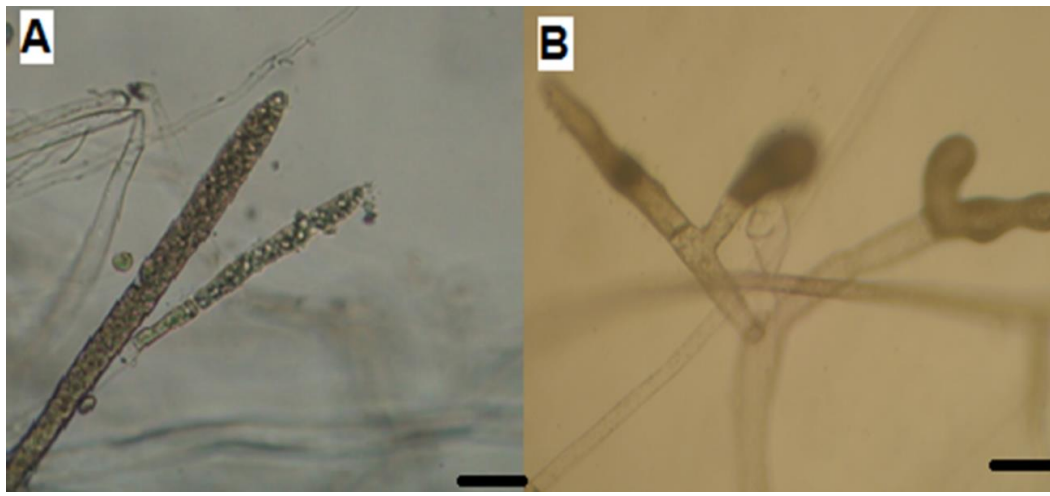

Fig. 1: Morphological features of fungus *Dictyuchus sterile*. A) dictyoid discharge mode of zoospores 7 days after inoculation in water culture; B) globular, terminal gemma. Magnification power 40X. scale bar =50 µm.

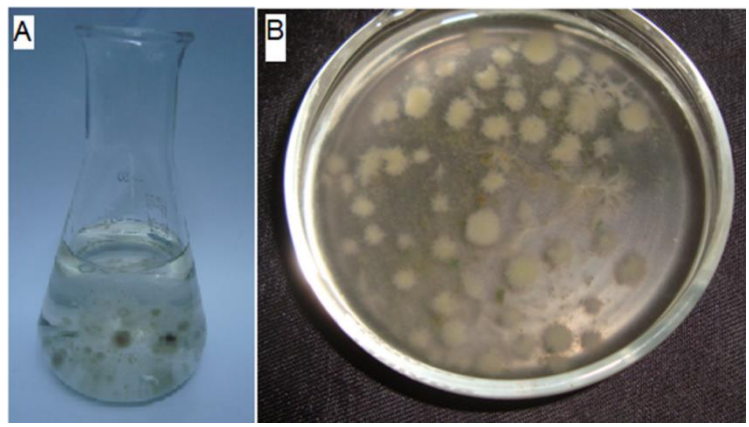

Fig. 2: Fungal pellets after 5 days of incubation and shaking incubation with 180 rpm 25°C.

Table 1: Incubation factors affect removal efficiency percentage and mass of adsorbed copper on the weight of fungal biomass adsorbent represented as the mean of three replicates ± standard deviation. Deferent letters within the column represent the significant differences ($p \leq 0.05$).

Factors		RE%	M [mg.g^{-1}]
Adsorption dosage of fungal pellets biomass [mg]	200	16	17.12±1.4c
	400	23	18.82±1.9c
	600	61	20.33±0.3b
	800	91	55.90± 0.8a
	1000	92	59.98±1.8 a
Contact time (mins)	15	11	±0.22c12
	30	13	±0.5c14
	45	67	±0.3b32
	60	88	±0.04a54
	75	91	±0.12a56
The initial concentration of copper ions [ppm]	20	94	±0.2a18
	40	91	±05b33
	60	92	±0. 4b51
	80	90	±0.024b 74
	100	93	±0.25a88
Shaking rate [rpm]	50	55	±3.37c 13
	100	54	±0.061c11
	150	76	±0.011c 43
	200	88	57±0.06b
	250	93	± 0.04a 59
pH	3	43	±0.10c 32
	5	92	57±0.2a
	7	66	41±0.1b
	9	21	11±0.2d
	11	13	9±0.3e
Temperature [°C]	20	32	31.55 ± 0.10c
	25	95	72.18 ± 1.02a
	30	88	54.56 ± 2.6b
	35	79	49.34 ± 3.37b
	40	34	18.55 ± 0.10c

vegetative structures, which are non-septate hypha. In Fig. 1 A, B, dictyoid and achlyoid zoospores are abundant in a fusiform and spherical shape, while the sporangia are abundant in branches, and sexual reproduction is absent (Fig. 2).

Incubation Conditions Affecting Copper Adsorption

Adsorption Dosage of Fungal Pellets

As shown in (Table 1), copper ions adsorption exhibits a clear relationship with the amount of fungal pellets biomass; as biomass quantity increases, copper ions adsorption increases (Bulgariu & Bulgariu 2012). An equilibrium has been formed, and ions-saturated connections are present since further increase has no discernible effect.

Initial Concentration of Copper Ions and Temperature

Due to easy access to binding sites, fungal biomass is overloaded; as the concentration of metal ions increases, the removal percentage decreases. The ratio of copper ions adsorption and equilibrium rises when copper ion concentration increases to 120 mg.L^{-1} because of the higher concentration gradient's force to startle. This demonstrates that copper ions have a better removal by keeping optimized parameters constant, such as 25°C, 1 gm.100 mL^{-1} dose for copper for 75 h, 180 rpm, pH 5.5, initial ion concentrations range from 20 to 120 mg.L^{-1}. Table 1 shows the dosage of fungal biomass effect on copper ion removal; at 120 ppm, the initial Cu concentration removal efficiency was 95%. In this investigation, the elimination present as biomass grows is directly associated with biomass dosage. As the concentration of copper ions in the solution grew, increasing removal percentages were seen until all of the adsorbent's binding sites were covered (Mathew et al. 2016). Once equilibrium is attained, the adsorbent's ability to remove metal ions decreases due to a lack of binding sites. It was discovered that the initial copper ion concentrations and clearance percentages were 95 percent at 120 ppm. Large amounts of fungal biomass can help clean up polluted aqueous solutions and restore polluted ecosystems. According to research, fungi can survive in temperatures as high as 40°C while feeding on various carbon sources, including fatty acids and oils. According to earlier research (Iskandar et al. 2011, Li et al. 2014), excellent efficiency is only seen at temperatures above 40°C. Therefore, fungi's ability to remove heavy metals in cold regions is insufficient.

Effect of pH on Adsorption

The most crucial component in ions adsorption is water pH, which also impacts the solubility of metal ions, the chemistry of metals in water, and the charge on the surface of cells (Bulgariu & Bulgariu 2012). According to Table 1, the pH range was between 3-11. The findings demonstrated that the chemical characteristics of an aqueous solution with a low pH and surface locations for metal adsorption on fungal biomass influence copper adsorption. Impact on the pH of the dissolved ions on the fungal cell wall's H_3O^+ bonding. Additionally, ions at low pH do not provide a positive metal to biomass due to the repulsive forces produced. In other words, the amount of negative load on these sites is decreased due to the high proton concentration and the associated shortage of protons in related sites. Therefore, it lessens or stops ions from bonding. When the units are released, the metal adsorbs to the surface due to the units' negative

charge. The chemical characteristics of each metal determine the pH of the cell.

Due to enhanced copper absorption, the ideal pH for the adsorption process in this investigation was chosen to be 5.5. These outcomes, which are displayed in (Fig. 4), came from the investigation of the adsorption. The study's findings about the morphologies of Oomycota indicated a consistent ability to adsorb trace metals from aqueous solutions (Kaczmarek & Boguś 2021). According to a report, the wall of filamentous fungal cells contains chitin, which is involved in the metal adsorption process (Shakya et al. 2016). Compared to chitin, which is a powerful N-poly adsorbent, chitosan (di acetate Acetyl glucosamine) it's thought to take traces of metal out of aqueous solutions. The amino group's nitrogenous location should cause this. The maximal adsorption capacity of the fungus is related to the amount of chitosan it contains because chitosan causes adsorption. The primary components of filamentous fungi's cell walls are chitosan, chitin, and phosphorous. However, a sizable portion of the filaments' wall is composed of chitin and chitin, and its quantity is higher than that of yeast (Hall 2002, Bellion et al. 2006). The increased capacity of the heads to absorb metal solution pH increase can be due to other components, such as galactose and lipids, which are present in equal concentrations. The adsorption of hydrogen ions might cause it from the aqueous solution that was either neutralized, or H^+ liberated from fungal biomass (Sen 2012).

Effect of Shaking Speed

With more incredible shaking speeds, metal ion adsorption rises. After examining several shaking rates, 250 rpm was determined to be the ideal speed, as shown in (Table 1). The largest amount of copper ions was found to be adsorbed at 210 rpm. In contrast, all other parameters, including temperature (25°C), dosage (1.0 gm.50 mL^{-1} for 75 h), rpm (100, 130, 160, 190, 210), pH (5.5), and starting concentration (100 $mg.L^{-1}$), were held constant.

Incubation Time

For copper and biomass from fungal pellets, the adsorption process was examined for contact times ranging from 15 to 75 h. According to investigations and the findings in Table 1, the total time needed for this part was 75 h. The amount exceeds the highest percentage of adsorption, and after the specified period, copper metal typically responds in 50 min. The proportion of adsorption with the adsorbent does not change significantly with time, nor does the amount of adsorption. Calculated at that moment was 73.38 percent. Due to the enormous number of vacant sites in the early days, it is possible to keep the other variables constant. *Aspergillus niger* was used in earlier experiments to remove copper. Its highest maximum removal capability was observed in 18 h with 25.2 $mg.g^{-1}$ Cu (Tsekova & Todorova, 2002). After 6 h of incubation, *Rhizopus oligosporus* absorbed mercury at a rate of 33.33 $mg.g^{-1}$ (Ozsoy 2010).

Analysis of Active Compounds By FTIR Spectra and SEM Imaging

To examine the active groups in the cell wall, either the blank adsorbent (fungal pellets only) or the fungal pellets plus copper ion, both of which are shown in (Fig. 3). The fungus is typically present in samples that were collected both before and after IR spectrum adsorption. Copper adsorption peaks were visible in the FTIR spectrum of fungal biomass pellets before copper adsorption at 3425, 3138, 1745, 1456.3, 1016, 1058, 960, and 819 cm^{-1}. These peaks were moderate before and after adsorption and showed a reduction in peak intensity. The peak in the fungal biomass sample before copper adsorption at 3425 cm^{-1} is assigned to O-H. It can be attributed to the C-H group containing lipids and phospholipids. The band at 1635 cm^{-1} is assigned to C=O. The band at 1456 cm^{-1} is related to carbonate carboxylate or methyl. The characteristic adsorption peak at 1078 cm^{-1} represents C-OH stretching. Si-OH and S-O are assigned to the bands at 960 and 819, respectively. Before copper adsorption, the sample's FTIR spectrum showed that the previously mentioned characteristic adsorption peaks were shifted to new positions: 3425, 2926, 1635, 1456.3, 1078, 1024, 960 and 819 cm^{-1} after copper adsorption by the fungal pellets' biomass. These results show the association of these functional groups in the adsorption of copper ions. The peak at 2926 cm^{-1} representing C-H stretching vibrations shifted to 3425 $cm^{-1,}$ which was assigned to the stretching vibration of N–H. The carboxylic acid (C = O) band at 1635 cm^{-1} is moved to a lower wavelength at 1624 cm^{-1}. The variation in the wave number reveals the involvement of the carboxylic acid in the ion exchange process. The peak at 1456 cm^{-1} representing C-H stretching vibrations shifted to the peak at 1422 $cm^{-1,}$ which is assigned to the stretching vibration of C = O. In addition, a peak at 819 cm1 shifted to 602 cm1 might be associated with the bending modes of alcoholic hydroxyl (-OH). The band moved from 1024 cm^{-1} representing C-OH stretching vibrations to 1078 $cm^{-1,}$ corresponding to C-O stretching vibrations.

In conclusion, FITR confirms that copper ions are adsorbed mainly by carboxylic, phenolic, and amide groups copper ions and the functional groups on the fungal pellets biomass complexed together, resulting in changes in wave numbers and intensities. The results of the spectra before the adsorption of copper metal in the normal state show that carboxyl functional groups- COOH, amide (NH_2),

3-phosphate $PO_{4,}$ and a hydroxyl group (OH) play an essential role in adsorption. Accumulation of copper on fungal surface morphology was examined by using SEM, extracellular aggregation of copper in the mycelial surface as in (Fig. 4), components of fungal cell walls as cellulose, melanin, and phenols represent sites for adsorption to occur, which provide oxygen-rich metal-binding (Garcia-Rubio et al. 2020). These cell wall components (exopolysaccharides) could be most possibly responsible for the adsorption of heavy metals (Liu et al.2001).

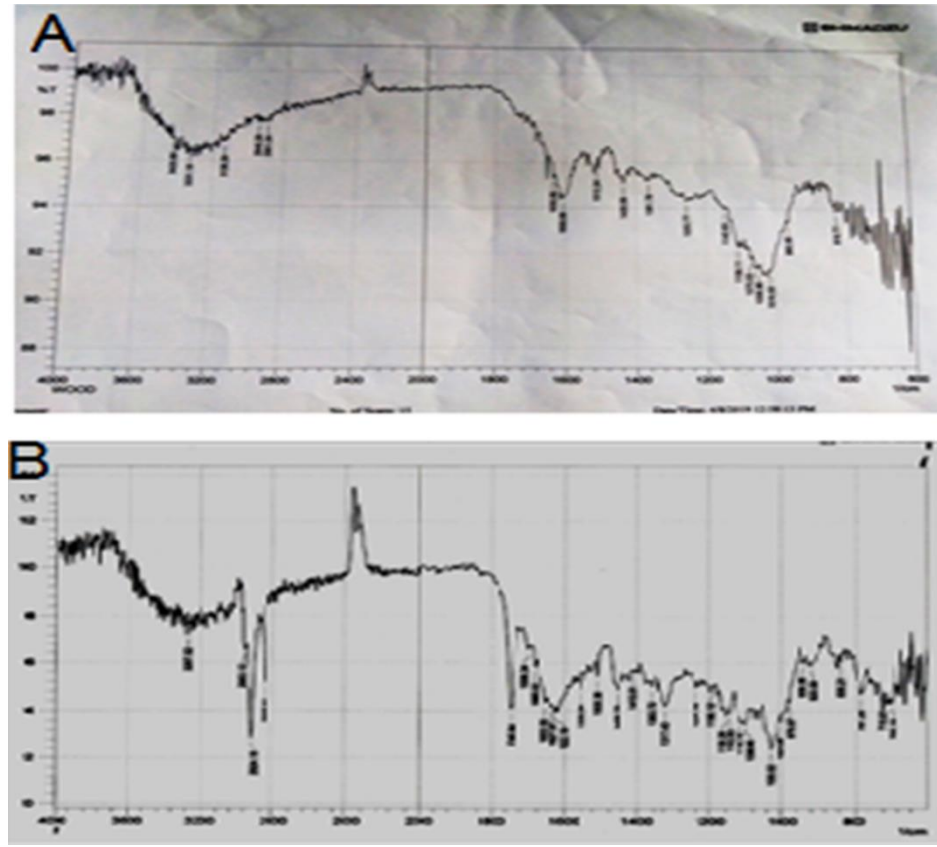

Fig. 3: Fourier transforms infrared spectroscopy(FT-IR) spectra of fungal pellets. A) before copper adsorption and B) after copper adsorption.

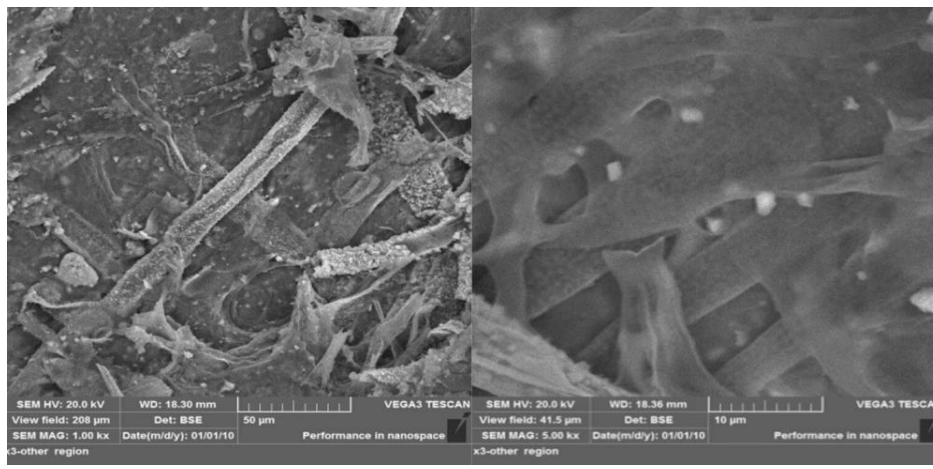

Fig. 4: SEM micrograph of the fungal pellets grown at 100 ppm copper.

CONCLUSION

Fungal biomass pellets of *Dictyuchus sterile* were used to remove copper metal from the aqueous solution. The highest removal percentage of copper was 95 %, and fungal pellets adsorbed copper. Fungal biomass pellets of D. sterile (M) was 57 $mg.g^{-1}$ were established at optimized conditions: pH 5.5 with 75 h of contact time during the experiment, one biomass dose of fungus pellets was used, a 100 ppm concentration was used, 250 rpm was used, and the temperature was 25 °C. According to this study, removing heavy metals like copper from contaminated water using the intended method is a more environmentally friendly option. According to FTIR spectrum analysis and SEM images, the principal adsorbents for copper ions on the adsorbent surface are active groups in the fungal cell wall like carbonate, hydroxyl, carboxyl, phosphate, and ammonium nitrate.

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