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IMMOBILIZATION OF *SAPROLEGNIA FERAX* IN DATE PALM FIBRILLIUM AS BIOLOGICAL FILTER FOR POLLUTED WATER WITH HEAVY METALS

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Abstract

One of the most important types of pollution threatening the aquatic environment is heavy metal pollution in aquatic ecosystems. As a method was adopted to immobilize the aquatic fungi isolated from the study area in the date palm fibrillium for removing these heavy metals such as (copper, iron and lead) from their aqueous solutions. A study was also conducted on immobilize fungi with the possibility of using biomass to get rid of heavy metals that are pronounced with the wastewater left behind some industrial activities. A study on the bio-remediation of minerals by resistance fungi, by immobilize them to palm fibrillium and then the interference between fungi and wood fibrillium was demonstrated through an electron microscopy image analysis system and the work of FTIR examination indicates the presence of bonds and effective groups as it was revealed the presence of multiple sugars such as ketene and chlorane entering. In the synthesis of hyphal walls as it contains the amine aliphatic group N-acetylglucoseamine (1030.02 cm^{-1}), galactosaminogalactan and a phosphate group (1234.48 cm^{-1}) and polysaccharide compounds (1030.02 cm -1 to 1143.83 cm^{-1}), a noticeable increase was found in the concavity of the active group area NC (1022.31 cm^{-1}).

Key words: aquatic fungi, date palm fibrillium, bubble column reactor, Biosorbent, Wastewater treatment.

Introduction

Water resources in any country are the most significant foundations of national wealth, it is the mainstay of life and one of the most important elements of life in most countries of the world. In Iraq, home to two great rivers, the word "Iraq" has become synonymous with "Mesopotamia" There is no doubt that agricultural and industrial operation, household uses and wastewater, particularly hospitals near water sources, all have an impact on the quality of water and aquatic life, as well as on the use of fertilizers, pesticides and herbicides in agriculture (WH0, 1996). The Euphrates river is one of the major rivers influenced by a number of human activities and industrialization that which contributed to the introduction of heavy metals and other contaminants into the ecosystem (Gupta *et al*., 2014; Shah, 2014; Bhattacharya *et al*., 2015). Industries discharge heavy metals containing waste directly into the river and drain through vegetables irrigated with contaminated water into the food chain (Nguyen *et al*., 2013).

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Heavy metals must be extracted or transferred to less toxic forms in sewage before disposal to the environment or before irrigation chemical and Physical processes are conventionally used to remove heavy metals, but these methods are not cost-effective when the concentration of heavy metals is very small (Li *et al*., 2013). Alternatively, the biological method using living organisms like fungi is explored (Avery and Tobin, 1993). Fungal species are known for absorb metals efficiently, either through biosorption or bioaccumulation, Biosorption occurs as metal ions are sequestered to the cell wall by surface bonding during bioaccumulation, metal ions are intracellularly assimilated (Huang *et al*., 1990; Brady *et al*., 1994).

Materials and Methods

Description and samples collection of the study area

The samples were collected from five sites in the river between Daghghara river through Sinniyah district to the AL-Diwaniya city. Five stations were collected water samples during April, 2019 which are:

Station 1 is located to the north of the city center, the river at this station is surrounded by some agricultural land and is characterized free of aquatic plants on both sides of the river.

Station 2 is located in the city center; It is characterized by the presence of many pollutants that are received directly in the water of the river and contains many aquatic plants on both sides of the river.

Station 3 is located south of the city, characterized by a lack of aquatic plants on both sides of the river.

Station 4 is located between the end of Al-Diwaniyah river and the beginning of Al-Muthanna.

Station (5) is located in the east AL-Diwaniyah, were its characterized the density of vegetation and agriculture.

Isolation of *Saprolegniaceae* genera from water

Water samples have been isolated by Baiting technique (Jones, 1971) and sesame seeds have been used for this purpose, as the study (AL-Rekabi *et al.*, 1996) confirms the efficacy of the use of these seeds in addition to Cannabis seeds in both grafts and traps in the insulation of water molds. After shaking, gently add 1 ml of water in 10 cm Petri dishes containing 10 ml of sterile distilled water and (1-4) sterile sesame seed by boiling for 15 minutes. In order to eliminate bacterial contamination, an optimal concentration of 3 mL of chloramphenicol solution prepared by dissolving 250 mg of chloramphenicol in 250 ml of warm sterile distilled water was found to eliminate this contamination. These Petri dishes were incubated at 20°C and examined by a light microscope after 48 hours to monitor the growth of undivided fungal filaments. On sterile distilled water, chloramphenicol and new seeds remained at the same temperature for a few days until the fungal hyphae elongated. They can separate and prepare pure cultures from them. After a week, dishes that did not show growth were neglected.

Diagnosis of aquatic fungi

Diagnosis of aquatic fungi based on a method described by Seymour, (1970), which included a focus on the morphological characteristics of vegetative and sexual reproductive structures in aquatic environments.

Immobilization of *Saprolegnia ferax* on fibrillium

Five to ten of fibrillium pieces were transferred to 100 ml of Potato Dextrose Broth medium in 250 ml flasks. Each flask was inoculated with spore suspension 1×10^4 of *S. ferax* and incubated in shaken condition (120 rpm) for 4-7 days. The pieces immobilized with fungal cultures were harvested from the culture media and oven dried at 60°C for 48 h.

Reactor design

The reactor is an open column with a downward air inlet and two side outlets. The reactor in (Fig. 1a) schematic diagram in operating condition. The immobilized *S. ferax* on date palm fibrillium were able to disperse evenly throughout the entire column and white broad arrows indicated their general flow directions. (Fig. 1b) photo of reactor in real so that the processed wastewater could be discharged through the outlet 1 after pellet settlement. Upon completion of all batches, outlet 2 was used for complete discharge.

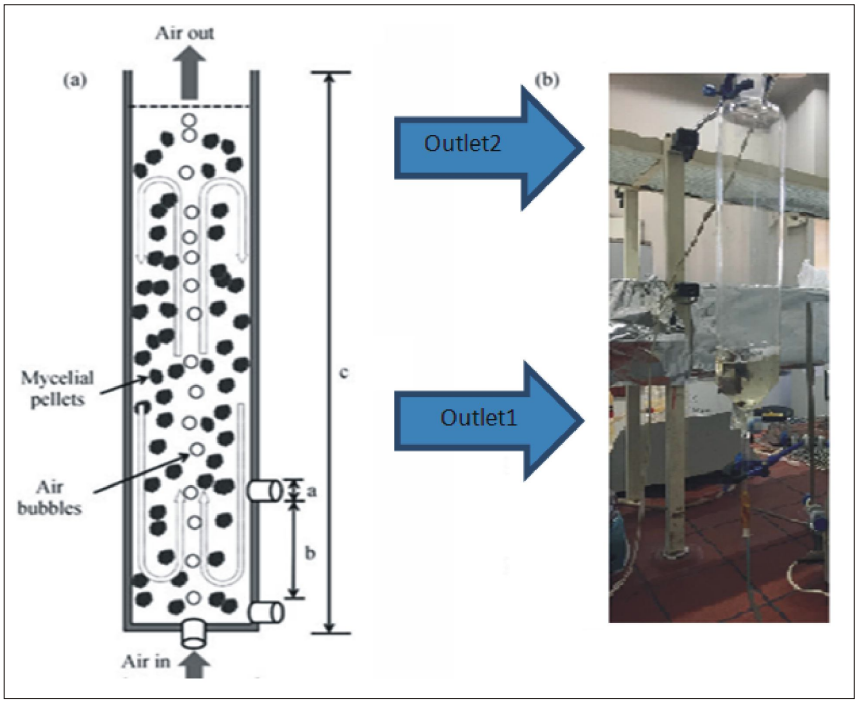
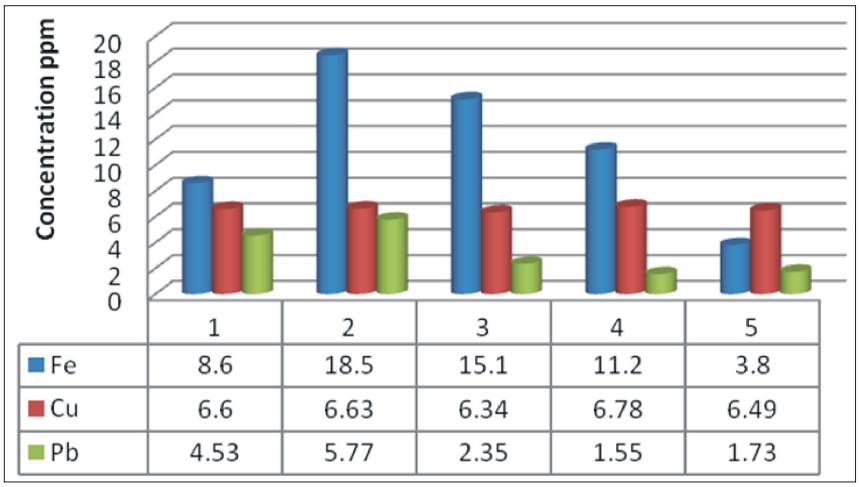


Fig 1: The Schematic diagram of the (a) running and (b) resting status of the bubble column reactor. The dimensions are (cm): a, 1; b, 9;c, 40; d, 6.5. The air inlet and two outlets are the same in size.

Removal of heavy metals in reactor

A design of semi-continuous bubble column reactor was previous sketched to meet the operation of repeat use of immobilized *Saprolegnia ferax* in fibrillium. The reactor was local made in Mustansiriya University. Batch studies were conducted to validate the feasibility of the reactor. In the first batch, 500 mL tap water containing (30 g wet weight) of immobilized *Saprolegnia ferax* in fibrillium and deferent concentrations of heavy metals (500, 250, 100) ppm for each heavy metal were added into the



	1	2	3	4	5
Fe	8.6	18.5	15.1	11.2	3.8
Cu	6.6	6.63	6.34	6.78	6.49
Pb	4.53	5.77	2.35	1.55	1.73

Fig. 2: The concentration of metals in water before treatment in ppm.

bubble column reactor. The reactor was kept at 25°C, 1.0 L/min of aeration for 12 h.

$$\text{Heavy metal removal equation (\%)} = \frac{Ci - Cf}{Cf} \times 100$$ (YU *et al*., 2019)

Characterization

SEM (Tescan VEGA3, Japan) was used to demonstrate distribution of the fungal hyphae on the date palm fibrillium. Dried date palm fibrillium and date palm fibrillium contains growing fungus were scanned by FT-IR 8400S, shimadzu (Japan) from 3500 to 800 cm^{-1} to analyze their chemical structures.

Results and Discussion

Iron, Copper, Lead concentration in study area

The results of the study indicate that It appeared that the Diwaniyah river was polluted with these elements above the permissible limits for classification of water pollution (WHO, 2015). Reaching the maximum concentrations of the measured elements respectively (18.5, 6.63, 5.77) ppm. Fe was the most common element in all stations, but the second was more polluted by elements. There are several reasons of water pollution with elements either from the rocks surrounding the river. According to (AL-Juboury, 2008) Lead element had its value at all stations (4.53, 5.77, 2.35, 1.55, 1.73) ppm. This study is consistent with (AL-Taee *et al.*, 2007) for the water of the Hila river the increase in Pb concentration in the aquatic environment is caused by domestic uses, sewage and plant waste the station (2) was close to a group of factories that dump their waste directly to the river without treatment so it appeared more than other stations that's agree with (Ali, 2007). About Cu element had its value at all stations (6.6, 6.63, 6.34, 6.78, 6.49) ppm. Its presence in the Diwaniyah river was high and this was agree with his findings (Hamid, 1998) of the Diyala river and the study (Hussein *et al*., 2007) The Hilla river, which is one of the main heavy metals and the most important sources of pollution of Cu is industrial waste because it is widely used in industry.

Fe element had it value at all stations (8.6, 18.5, 15.1, 11.2, 3.8) ppm. A laboratory study was conducted to study the effect of this element on the physiology and growth of some aquatic fungi. The Diwaniya river is polluted with iron, according to (WHO, 2015) because the limit is 0.3 mg/l. Statistically, the study significant differences for each of the elements with the studied sites, as they were valuable that's mean concentration of these elements varies from station, especially near diesel generators are produced, through which the dust can transfer as it is deposited on the surface of the nearby, the values can be observed ($P < 0.05$) a significant relationship was achieved in increasing concentrations with proximity and distance from source of pollution [Mean Fe (11.44) P value 0.04] [Mean Cu (6.57) P value 0.01] [Mean Pb (3.91) P value 0.02] (Fig. 2) as following show different concentrations relative in plants to the five sites.

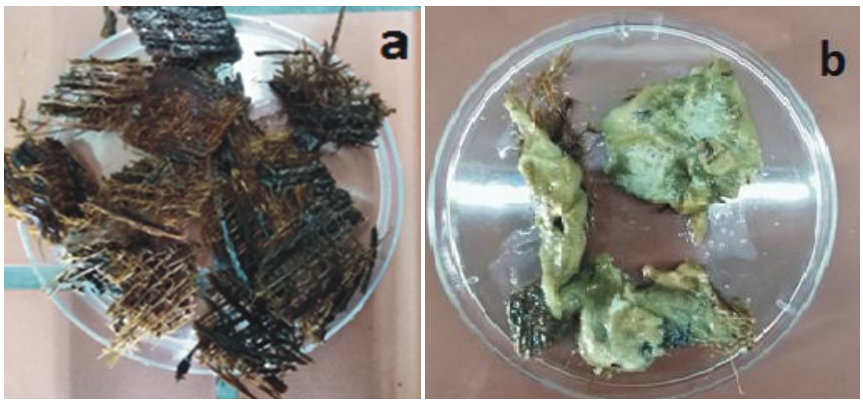


Fig. 3: (a) Date palm fibrillium partsa: (b) before fungi immobilization.

Isolated aquatic fungi of this study

Fourteen isolates of aquatic fungi were obtained and five species belonging to oomycites fungi were isolated and isolated fungi were classified into four genus, three of which belong to order the Saprolegniales of the Saprolegniaceae family, they are Achlya, Dictyuchus, Saprolegnia and the one genus is of order Peronosporales of the Pythiaceae family, the Pythium genus

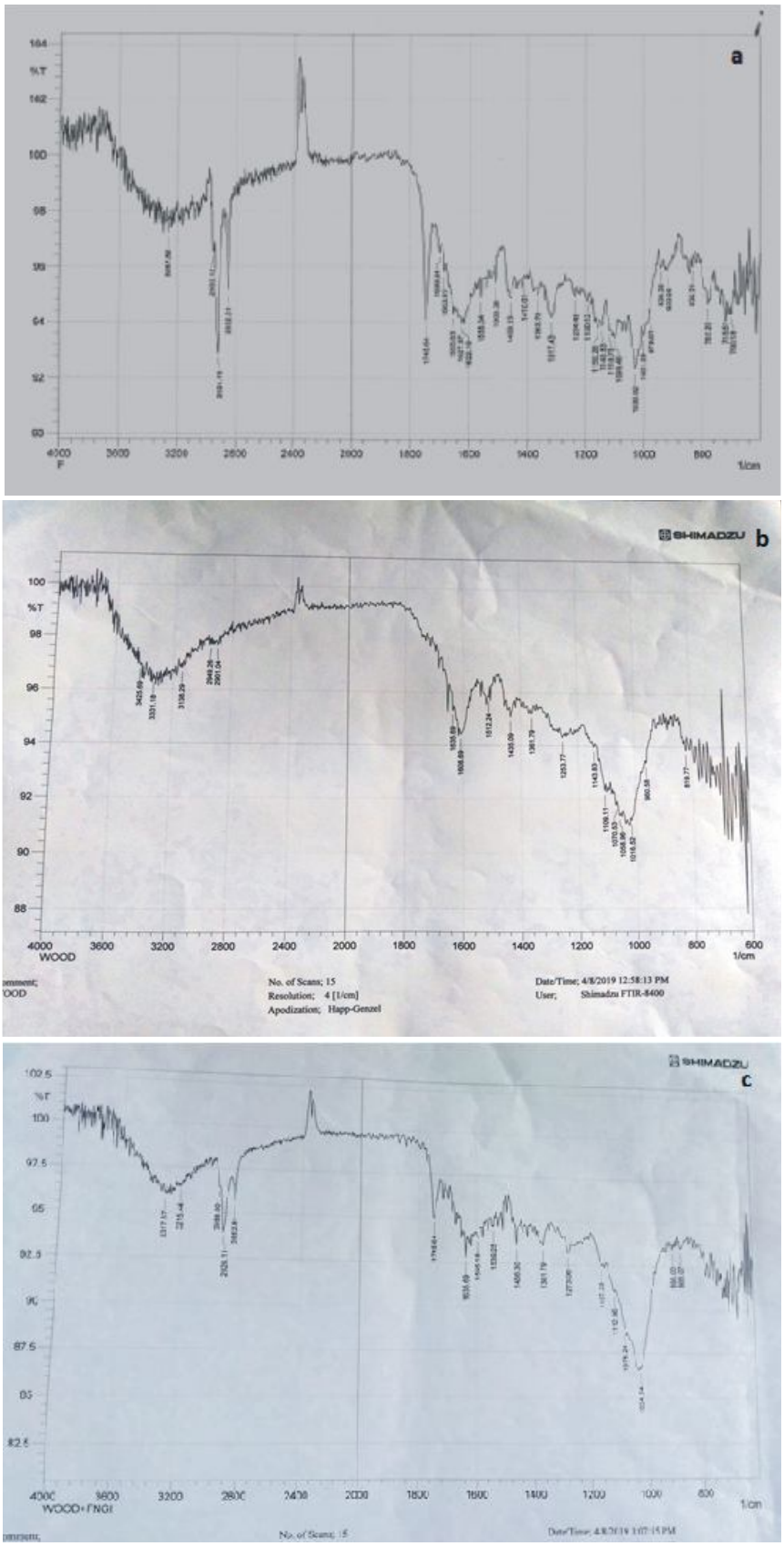


Fig. 4: FTIR spectra of immobilized *Saprolegnia ferax* on fibr illium ,a : *S. ferax* mycelium before treatment ,b: date palm febrillium before treatment, c : immobilized *S. ferax* on date palm febrillium after treatment.

and the samples (isolates) belonging to the two genus Dictyuchus, Pythium could not be diagnosed at the gender level because they did not form the sexual phase.

The diagnosed species included: *Achlya americana*, *A. klebsiana*, *Dictyuchus sterile*, *Pythium* sp., *Saprolegnia* sp., *S. ferax*.

The result showed the highest adsorption rates on the last day of Iron that (30.43ppm) copper that (212.26 ppm) and lead that (6.59ppm). They are found on the cellular wall level of the fungus is a group of ligand. It caches minerals, which includes totals both carboxyl, amine, hydroxyl, phosphate and sulfhydryl. Elements can be they are adsorbed by forming complexes with negatively charged reaction centers that are present at the cellular surface leave (Gupta *et al*., 2000). The relative importance and role of each functional group is often difficult select it There are differences in the harmony of both elements and their ionic types with ligand found in bailout systems. These differences are shown above in the table where they showed that the Percentage absorption of iron at last day that 61% was different from lead that 39% and copper 43%.

According to the above results correlation between the adsorption ratio of each element to the probability (note the probability and not applicable results) means if distilled water was contaminated with the Iron, lead and copper elements the removal rate was 61% for iron and 43% for the copper and 39% for lead how much will be the proportion of natural water river water. When making the correlation probability between the last day and percentage absorbent with fungi, you will notice that the lead removal rate is higher than that of iron and copper if it was river water. From the results it is clear that the fungus is more effective in the removal rate of the element lead and this may have a positive indicator for the use of this type

Table 1: Shows each element with the last day of treatment and this ratio is the ratio of fungus adsorption with the elements.

	Fe last day	Cu Last day	Pb Last day
Last day	30.43	212.26	6.59
Mean all day	54.92	200.91	5.91
Percentage absorption	61%	43%	39%

of experiments in water artificially contaminated with the elements, especially the element lead.

Bio-reactor of immobilizes *Saprolegnia ferax* on date palm fibrillium

The three- dimension network made of lignocellulosic hollow microfibers is highly porous and therefore gives rapid and wide contact between its surface and adsorbed substances. This material was exploited in our present study. Date palm fibrillium collected from local farms and cut into pieces (1 ×1 cm) (Fig. 3a). The flasks containing growth medium along with fibrillium pieces were inoculated with spore suspension (1×10^4) of 4 days old *Saprolegnia ferax* culture incubated in shaken condition. The growth hypha on fibrillium pieces were monitored for 3-6 days. The porous fibrillium pieces did not have any negative influence on the growth of these fungal organisms. The mycelia of fungi showed rich growth proliferating on the entire surface and porous part of the fibrillium pieces forming a cushion shaped structure at the end of the growth period on the day 6 (Fig. 3b). After the incubation period, the immobilized fungal biomass was gently removed from the growth medium and dried at 60°C for 24 h. and used as a biosorbent material for scavenging ions.

Identification of functional groups in immobilized fungi for biosoprption of elements

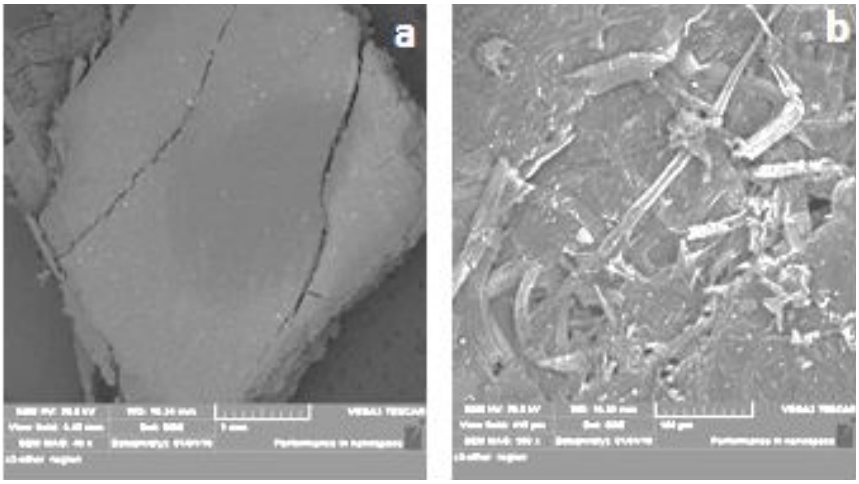


Fig. 5: SEM micrographs showing surface morphology of (a) structure of date palm fibrillium , (b) date palm fibrillium immobilized with fungi, and before biosorption.

Table 2: Compared with the adsorption results and percentages obtained and find Coloration Probability.

	Fe	Cu	Pb
Mean Station 2	18.5	6.63	5.77
Percentage absorbent with fungi	61%	43%	39%
Coloration Probability	**31.60%**	**25.70%**	**54.80%**

The FTIR spectra of the biosorbent before and after adsorption of heavy metals (Table 3) depicts the functional groups responsible for metals adsorption (Fig. 4 a,b,c). The concavity around 1024 cm^{-1} increased to 1016 cm^{-1} after biosorption. This suggests that the free carboxyl group has transformed into carboxylate group, a phenomenon which normally occurs during the reaction between metales and carboxyl group indicating site specific interactions according to (Yu *et al*., 2019) use the microorganism Arthrobacter. ZXY-2 exhibits excellent degradation efficiency for atrazine in free cells. However, its poor fixability makes it hard to be kept and recycled in water. To conquer the problem, this work employed mycelial pellets of *Aspergillus niger* Y3 to immobilize ZXY-2, which formed a self-immobilized biomixture (SIB) to remove atrazine. It is well documented that *Saprolegnia ferax* contains cell walls formed by chitin molecules –long chain polymer of N-acetylglucosamine. The identified functional groups, –OH, –NH and –CH 3, in our study provide further evidence to support this statement since these groups are among few of the main constituents of N- acetylglucosamine. Bhanoorinad and Venkateshwerlu (Sriharsha *et al*., 2017). Also reported previously that the oxygen ring and the hydroxyl groups of N-acetylglucosamine plays a significant role in binding of lead ions via complexation. The shift provides an indication that the acidic groups, carboxyl and hydroxyl groups are predominant contributors in the chitin-lead complex.

Adsorption of heavy metals on immobilized fungal biomass-Scanning Electron Microscopy studies

Additional evidence was drawn on the adsorption of heavy metals by the adsorbent under Scanning Electron Microscopic observation. SEM was performed at the Technical University / Production and Minerals Department.samples were gold-coated by cathodic spraying and observed under a scanning electron microscope. The uniform distribution of the fungal hyphae on the date palm fibrillium is one

Table 3: The functional groups involved in biosorption.

Number of group	Wavelength (cm-1)	Bond	Functional group
1	3500-3200	O-H	Alcohol, Phenols
2	3000-2800	C-H	Alkanes
3	1700-1450	C-C	Aromatic
4	1300-1100	C-H	Alkanes
5	1000-800	C-H	Aromatics

of the important criteria for the proper biosorption of the metal ions on the entire surface area of the immobilized fungal cells. The untreated plain date palm fibrillium depicted lengthy, thick fibre radiating across to each other (Fig. 5a). Before the adsorption process, the designed date palm fibrillium with immobilized fungal biomass significantly showed thick and uniform growth of fungal mycelia covering the entire surface of the fibrous network of date palm fibrillium (Fig. 5b). After adsorption process, the adsorbents surface.

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