www.biodicon.com Biological Diversity and Conservation

ISSN 1308-8084 Online; ISSN 1308-5301 Print 12/3 (2019) 44-56

Research article/Araştırma makalesi
DOI: 10.5505/biodicon.2019.42714

Biosorption of cadmium and copper by *Aspergillus* spp. isolated from industrial ceramic waste sludge

Elif HASGÜL[1], Semra MALKOÇ [*1], Alaettin GÜVEN[3], Alper DEDE[4] , Kıymet GÜVEN[4]
ORCID: 0000-0002-0424-4470; 0000-0002-8092-411X; 0000-0003-2683-1008; 0000-0002-0707-9781; 0000-0001-6683-0419

[1] Eskişehir Osmangazi University, Graduate School of Sciences, Eskişehir, Türkiye
[2] Eskişehir Technical University, Engineering Faculty, Environmental Engineering Department, Eskişehir, Türkiye
[3] Eskişehir Technical University, Science Faculty, Chemistry Department, Eskişehir, Türkiye
[4] Eskişehir Technical University, Science Faculty, Biology Department, Eskişehir, Türkiye

Abstract

Under proper conditions, fungi can act as a good biosorbent for different heavy metals. In the present study, *Aspergillus* spp. have been isolated from ceramic industrial waste sludge and the tolerance of the fungi for copper and cadmium metals were examined. The experiments were carried out at 25 °C, pH=4-4.5 for Cu(II), pH=6 for Cd(II), biosorbent dose of 2.5 g, initial metal concentration of Cd (II) was 1 mM and Cu(II) was 5 mM. The removal efficiencies for cadmium and copper with two *Aspergillus* strains were found to be 90-95% and 85-90%, respectively. The sorption capacities of live and dead fungi for copper were 5.3676 mg g^{-1}, 18.661 mg g^{-1} and for cadmium were 0.1977 mg g^{-1}, 0.1772 mg g^{-1} respectively. FTIR analyses have showed that copper ions bound to vinyl compounds (950-900 cm^{-1}) and cadmium ions bound to primer amides (1420-1400 cm^{-1}), mostly. Considering biosorption results, Langmuir and Freundlich isotherm models have been described and it was clearly seen that none of the isotherm models have fitted the experimental data. The metal ion binding areas of the cell surface of fungi were determined by FTIR. SEM monitoring and EDX analysis were carried out. EDX results confirmed the biosorption of copper and cadmium.

Key words: *Aspergillus* spp., cadmium, copper, biosorption, ceramic industry

---------- * ----------

Seramik endüstrisi arıtma çamurundan izole edilen *Aspergillus* spp. ile kadmiyum ve bakır biyosorbsiyonu

Özet

Küfler uygun koşullar altında farklı ağır metaller için iyi bir biyosorban olabilir. Bu çalışmada, seramik sanayi atık çamurlarından *Aspergillus* spp. izole edilmiş ve bakır ve kadmiyum metallerine toleransı incelenmiştir. Deneyler 25 °C'de biyosorban miktarı 2,5 g, Cu (II) için pH = 4-4,5, Cd (II) için pH = 6 ve başlangıç metal konsantrasyonu Cd (II) için 1 mM ve Cu (II) için 5 mM olarak yapılmıştır. İki *Aspergillus* suşunun kadmiyum ve bakırı uzaklaştırma etkinlikleri sırasıyla % 90-95 ve% 85-90 olarak bulunmuştur. Canlı ve ölü biyosorbanın bakır için emilim kapasiteleri sırasıyla 5,3676 mg g^{-1}, 18,661 mg g^{-1} ve kadmiyum için ise sırasıyla 0,1977 mg g^{-1}, 0,1772 mg g^{-1} olarak tespit edilmiştir. FTIR analizleri bakır iyonlarının vinil bileşiklere (950-900 cm^{-1}) ve kadmiyum iyonlarının primer amidlere (1420-1400 cm^{-1}) bağlandığını göstermiştir. Biyosorpsiyon sonuçları dikkate alındığında, Langmuir ve Freundlich izoterm modellerinden hiçbirinin deneysel verilere uymadığı açıkça görülmüştür. Hücre yüzeyinin metal iyon bağlama alanları FTIR ile belirlenmiştir. SEM izleme ve EDX analizi yapılmış, EDX sonuçları bakır ve kadmiyumun biyosorpsiyonunu doğrulamıştır.

Anahtar kelimeler: *Aspergillus* spp., kadmiyum, bakır, biyosorbsiyon, seramik endüstrisi

[*] *Corresponding author* / Haberleşmeden sorumlu yazar: Tel.: +905323358248; Fax.: +902223239501; E-mail: satik@eskisehir.edu.tr

1. Introduction

Advances in industrialization that connected with the increase of people's needs have increased the amount of wastewater and heavy metal pollutions. Mines, petroleum refineries, metal industry, galvanic processes, ceramic industry, paint and plastic material productions are some of the main sources that contributing to heavy metal pollution [1]. Due to their properties, heavy metal ions have been used in many different areas of industry. However, metal ions can accumulate in living tissues and cause toxic effects in metabolism, without getting noticed. This case adversely affects both the environment and the human health [2]. Even small amounts of these heavy metals such as antimony, lead, silver, arsenic, cadmium, copper, nickel, iron, aluminum, cobalt, chromium, manganese, mercury, zinc, barium etc. might be toxic [3]. For example, public health goal are 0.006 mg/L antimony, 2 mg/L barium, 0.005 mg/L cadmium, 0.1 mg/L chromium (total) 1.3 mg/L copper, 0.002 mg/L mercury (inorganic) and arsenic and lead zero [4].

Various chemical and physical processes have been applied to remove of heavy metals from the industrial wastes. However, these methods are not economical always; in that case, industries tend to using microorganisms that have significant potential for removal of heavy metals [5]. A variety of bacteria, fungi and algae has been used for this purpose. Some living or dead microorganisms can adsorb the heavy metal ions. The microorganism, which will be used for removal of metals, should be able to easily and economically produce in high quantities [6]. Almost all organisms have negatively charged surfaces and they capable to adsorb positively charged metal ions, such as Cu^{2+}, Pb^{2+}, Zn^{2+}, Mn^{2+}, Cd^{2+}, Ni^{2+}, Hg^{2+}, Cr^{3+}, Cr^{6+}, Fe^{2+}, Fe^{3+} etc. Some living organisms take and accumulate the metal ions into the cell. Removal of heavy metals by biosorption is the result of an interaction between the cell wall and metal ions [7].

In the composition of ceramics, there are different types of silicates, aluminates, water, metal oxides, alkali and alkaline earth compounds. Ceramic is obtained by mixing some ingredients as natural clay, kaolin, quartz and feldspar in specific proportions [8]. Production processes in the ceramic industry generally involve the parts as design, slurry preparation, foundry, drying, glazing and firing units. In this kind of industries, the major sources of waste arises from sludge unit, mold section, foundry, glaze preparation and washing part [9]. Wastes from slurry preparation unit contains high amounts of suspended solids and organic contents which resulting excessive pollution load [10].

The aim of this study was to perform biosorption processes for removal of copper and cadmium metals by using fungi isolated from local ceramic industrial waste sludge. The metal binding sites on fungal cell surfaces were identified for both metals by using FTIR. SEM monitoring and EDX analysis were also carried out for determination of the accumulations of metals on fungal cell.

The relationship between the amount of solute adsorbed at equilibrium onto the adsorbent and the amount of remaining solute can be described by adsorption isotherms. The Langmuir and Freundlich isotherm models are commonly used for the description of adsorption processes [11].

2. Materials and methods

2.1. Isolation and identification of fungi

Ceramic waste sludge sample was obtained kindly, from a ceramic factory located very close to Eskisehir. The subsamples were taken from different locations of waste sludge tank by using a plastic shovel. The working sample was a mixture of these subsamples. Analysis of heavy metals in the sludge was carried out by ICP-OES (VARIAN 720 ES). The sample was prepared according to EPA 3051A method [12]. Briefly, the sludge was dried for 3 hours in the drying oven (Nüve FN 500) at 105 °C. A sample of dried mud have been prepared and combusted in microwave heating unit (CEM Mars Express) for ICP analysis. For isolation of fungi, 5 grams of sludge samples were added (under sterile conditions) into three flasks containing 5 mL of 0.1% sterile tetra-sodium diphosphate decahydrate (pH 7.0) and then were shaked for 30 minutes at 180 rpm. The sludge suspensions were diluted by 10-fold and spread on the surface of PDA medium. The plates were incubated at 28 °C for 7-10 days [13] and then examined for colonies with different morphologies and colors. Different colonies of fungi were selectively re-cultivated on PDA and incubated at 28 °C temperature for 7-10 days.

Pure cultures of fungi were examined morphologically by microscopy (Olympus C011) and identified with the help of [14-17].

2.2. Screening of resistance to heavy metals and determination of MIC

Copper ($CuSO_4.5H_2O$) and cadmium ($CdSO_4.8H_2O$) [13] were used for determination of minimum inhibition concentrations (MIC) by fungi isolated from the ceramic industrial waste sludge. A general fungal growth medium (PDA) was prepared and amended with various amounts of heavy metals concentration ranging from 1 to 20 mM for screening the resistance to heavy metals. The mycelium disks of test fungi (10^6 CFU ml) were spotted in duplicate on heavy metal containing plates. Control plates used only PDA medium without heavy metals. To observe the growth of fungi on the spotted area, the plates were incubated at 28±1 °C for 7 days. Heavy metal tolerance was determinated as the heavy metal that inhibited visible growth of test fungus [18].

2.3. Biosorption assays

Cadmium and copper biosorption studies were carried out after screening tests by using the most resistant organism for each heavy metal. Both living and dead biomass were used in biosorption experiments. Two duplicates were prepared for each application. The fungi were firstly grown on PDA and then were transferred to PDB (potato dextrose broth) liquid medium for incubation by shaking incubator for 5-6 days (at 125 rpm, 25 °C). Fungal mycelia were filtered by vacuum filter (Diaphragm Vacuum Pump GM-0.5011) through 150 µm sterile filters. The biomass was absolutely washed with distilled deionized water to be in the clear residual growth medium and used immediately thereafter. Dead biomass was prepared by autoclaving 2.5 grams of biomass. Biosorption studies were carried out by adding either living or dead fungi into 50 mL of metal solutions by stirring at 125 rpm. The working concentrations of metals were chosen 5 mM for copper, 1 mM for cadmium. The studies were carried out by using different contact times as 24, 48, 72, 96, 120 and 168 hours. In each sampling time, 30 mL of the solution removed and centrifuged at 8000 rpm (Hettich Zentrifugen Universal 320-R) for 30 min to separate the biomass.

Following centrifugation, the supernatant was filtrated as above and then heavy metal concentrations of the supernatants was determined using a Varian 720 ES inductively coupled plasma optical emission spectroscopy (ICP-OES). The pellets were used for FTIR analysis and SEM-EDX studies. The percentage removal were calculated by the following equation:

$$Percentage\ removal = \frac{(Co - Ce)x100\%}{Co} \quad (1)$$

where C_0 and C_e were concentrations of metal ions (mg L^{-1}) in initial solutions and after biosorption [19]. The amounts of adsorbed metal ions per fungal biomass unit (mg metal ions/g biomass) were calculated by using the following equation [11]:

$$qe = \frac{(Co - Ce)xV}{m} \quad (2)$$

where q_e was biosorpted heavy metal amount per fungal biomass (mg g^{-1}), V was volume of aqueous phase (mL) and m was the amount of biomass (g).

2.4. Biosorption isotherms

Freundlich and Langmuir models were used for biosorption isotherm models. Freundlich isotherm model was used for describing the nonhomogeneous solid surface adsorptions [20].

$$Log\ qe = Log\ Kf + \frac{1}{n} Log\ Ce \quad (3)$$

where K_f was Freundlich sorption constant about the capacity, n was Freundlich constant about the sorption density. Langmuir isotherm model was used for describing the homogeneous monolayer adsorption assuming the adsorbent surface is similar in terms of energy [21].

$$\frac{Ce}{qe} = \frac{1}{q\mathrm{m}K\mathrm{L}} + \frac{Ce}{q\mathrm{m}} \quad (4)$$

where C_e was metal concentration in solution after adsorption (mg L^{-1}), q_e was adsorpted heavy metal amount per fungal biomass (mg g^{-1}), q_m was adsorption capacity of monolayer adsorption (mg kg^{-1}), K_L was Langmuir sorption constant (L mg^{-1}).

2.5. Fourier transform infrared spectroscopy (FTIR)

Fungal biomass was dried vacuum evaporator. 1 mg sample/100 mg KBr in order to prepare translucent sample disk and was analyzed by FTIR (Perkin Elmer Spektrum 100) at Anadolu University Department of Chemistry. FTIR studies were carried out in the range of 800–2000 cm^{-1} at room temperature. The absorption spectrum of unabsorbed dry fungal biomass was used as control for comparison with leading adsorbed biomass to investigate the functional groups of fungi relation to biosorption of metal ions.

2.6. Scanning electron microscopy (SEM) and energy dispersive x-ray (EDX) analysis

SEM micrographs and EDX spectra were taken for both control groups and heavy metal treated fungi samples. The dried and powdered samples were glued onto the stubs with conductive carbon strips. The stubs have been coated with a thin layer of gold by lining machine (30 mA, 1 min) on the purpose of enhancing image resolution. The samples were monitorized by FE-SEM microscope (JEOL JSM-6335F) (20kV, 300X)..

3. Results

3.1. The heavy metal content of ceramic waste sludge

The ICP-OES analysis was carried out for determining the heavy metal content of the ceramic waste sludge. The ceramic sludge contained 0.69 mg kg^{-1} cadmium, 7.89 mg kg^{-1} chromium, 3.95 mg kg^{-1} copper, 0.2 mg kg^{-1} nickel, 9.38 mg kg^{-1} lead, 8.29 mg kg^{-1} manganese, 0.5 mg kg^{-1} antimony, and 54464 mg kg^{-1} iron. Maximum for any single composite sample-TCLP are 0.10 mg/l antimony, 0.50 mg/l arsenic, 7.6 mg/l barium, 0.010 mg/l beryllium, 0.050 mg/l cadmium, 0.33 mg/l chromium (total), 0.15 mg/l lead, 0.009 mg/l mercury, 1.0 mg/l nickel, 0.16 mg/l selenium, 0.30 mg/l silver, 0.020 mg/l thallium, and 70 mg/l zinc [22].

Traditional ceramic materials are made of clay, silica, feldspar and kuvars. Ceramic waste sludge were involving high amount of iron (54464 mg kg^{-1}) and some other heavy metals probably originate from these ingredients [23].

However, iron removal researches have needed high concentrations of metal. [24-25] Ujile and Joel (2013) and Karthikeyan et al. (2005) have worked on physical adsorption of iron and they have found that the physical adsorption was easier and cheaper than biosorption for iron. Physical sorption results from the electrostatic and van der Waals interactions (forces). Different adsorption substrates may be used for this reason [26]. Chromium and antimony weren't dissolved easily in water and changes on pH have caused to precipitation. Filtration with special resins have been referred against biosorption for manganese removal, by the reason of being cheaper and easier [27]. For these reasons, iron, chromium, antimony, and manganese have not been studied for biosorption and bioaccumulation.

3.2. Isolation and identification of fungi

Totally 10 species of fungi were isolated from ceramic waste and and these fungi were identified as *Aspergillus* sp. (2 strains), *Penicillium* sp. (5 strains), *Mucor* sp., *Rhizopus* sp. and *Trichoderma* sp.

3.3. Determination of resistance to heavy metals and minimum inhibition concentrations (MIC)

All the tested fungi grew on the metal containing media indicating their resistance to heavy metals. However, *Aspergillus* sp. (strain 2) had the highest copper (Cu^{2+}) resistance as with *Aspergillus* sp. (strain 1) had the highest cadmium (Cd^{2+}) resistance among fungi tested. These two *Aspergillus* sp. strains also showed highest lead (Pb^{2+}) resistance. *Aspergillus* sp.1 and *Trichoderma* sp. were the most resistant fungi against Nickel (Ni^{2+}). The experiment results for determination of resistance to heavy metals and MIC were given in Table 1.

Table 1. Determination of resistance to heavy metals and minimum inhibition concentrations (MIC)

	Minimum Inhibition Concentrations (mM)			
Fungus	Cu^{2+}	Pb^{2+}	Cd^{2+}	Ni^{2+}
Aspergillus sp.1	10	15	5	5
Aspergillus sp.2	15	15	1	1
Penicillium sp.1	<0.5	10	<0.1	1
Penicillium sp.2	<0.5	5	<0.1	5
Penicillium sp.3	<0.5	1	<0.1	<0.5
Penicillium sp.4	<0.5	5	<0.1	1
Penicillium sp.5	<0.5	5	<0.1	1
Mucor sp.	5	10	5	1
Rhizopus sp.	10	5	1	1
Trichoderma sp.	1	1	0.1	5

Acquired MIC results have been compared and initial metal concentrations for biosorption studies were determined as 5 mM for copper and 1 mM for cadmium. Biosorption experiments were carried out for only copper and cadmium removal since MIC values obtained for lead and nickel were quite low comparing to literature [28].

3.4. Biosorption

Aspergillus sp. strain 1 was used for cadmium and *Aspergillus* sp. strain 2 was used for copper bioremoval. The copper removal were found to be 89.6% (96 h) and 86.7% (168 h); the cadmium removal were found to be 97% (168 h) and 83.5% (72 h) by using living and dead biosorbents, respectively.

The sorption capacities of living and dead fungi were 5.36 mg g^{-1} and 18.6 mg g^{-1} for Cu^{2+}; 0.19 mg g^{-1} and 0.17 mg g^{-1} for Cd^{2+}. The biosorption capacities of copper and cadmium by different microorganisms on literature were given by Table 2.

Removals of Cu(II), Ni(II), Cd(II), Zn(II) and Cr(II) by using *Beauveria bassiana* results for maximum metal removals had found 74.13%, 75%, 63.4%, 67.8% and 61.13%, respectively [29]. Cu^{2+} removal by *Aspergillus niger* studies obtained 99.6% removal [30]. The cadmium toxicity in a cadmium-tolerant strain of *Aspergillus foetidus* had reported earlier that the living fungal biomass could remove 79% of Cd^{2+} from the liquid media at 100 µM Cd^{2+} concentration [31]. A research by dead *Aspergillus terreus* (strain ATCC-20516) as a biosorbent for copper removal from aqueous solutions and they could reach 72% removal ratio [19]. A study had reported about cadmium removal by living *Saccharomyces cerevisiae* that had observed 77% removal [32]. The yields of removal had found to be 81% and 94% in a research that had been involving copper and cadmium biosorption by dead *Geobacillus* spp. [33].

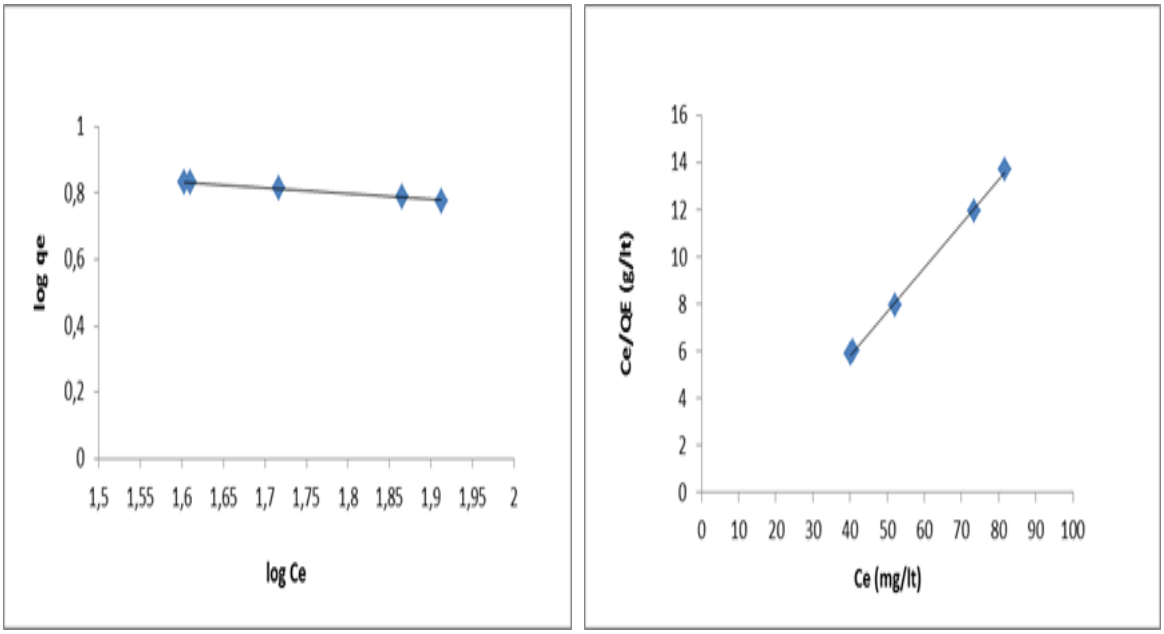
3.5. Biosorption isotherms

The sorption capacities have found for copper were 5.3676 mg g^{-1}, 18.661 mg g^{-1} and for cadmium were 0.1977 mg g^{-1}, 0.1772 mg g^{-1} in order of living and dead fungi. The Langmuir isotherm constants (K_L) have obtained in negative values. A negative value for the intercept of linear form of Langmuir model is physical nonsense, in other words it is impossible. As seen on Fig.1 and Fig.2, our Langmuir isotherms were considerably linear. Slopes of Freundlich isotherms (1/n) for Cu^{2+} and Cr^{2+} were also negative. For this reason, neither Langmuir nor Freundlich isotherm models were fitted our experimental data. The best way would be to perform a non-linear regression on our data, to use another isotherm model or work with higher initial metal concentrations. The sorption capacities (q_m), isotherm constants (K_L, K_F), coefficients of correlation (R^2) of isotherm models were given in Table 3.

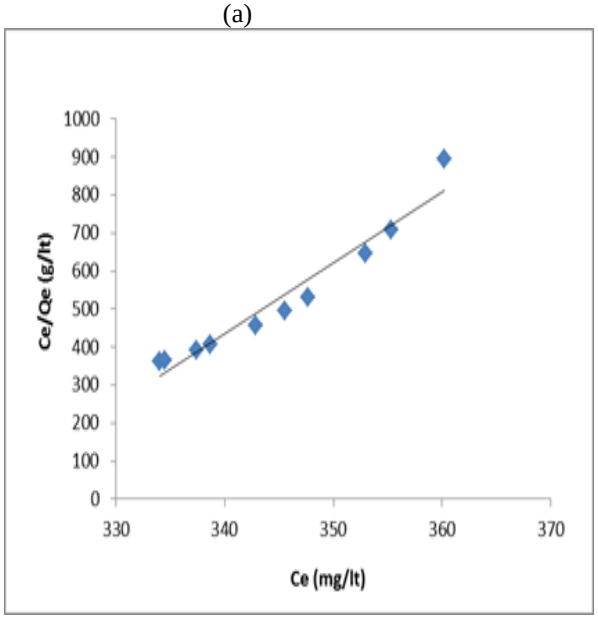
Table 2. Comparison the features of biosorption studies

Metal	Microorganism	pH	Temperature (˚C)	Sorption Capacity	Reference
Cd^{2+}	*A. foetidus**	5.0	32	73.46±10.75 mg g^{-1}	[31]
Cd^{2+}	*S. cerevisiae**	5.0	28	106.81 ± 3.71 µmol g^{-1}	[32]
Cd^{2+}	*Z. rouxii**	5.0	28	33.83 ± 1.25 µmol g^{-1}	[32]
Cd^{2+}	*A. symbioticum* H8**	6.0	30	248.62 mg g^{-1}	[34]
Cd^{2+}	*B. subtilis***	5.92	45	251.91 mg g^{-1}	[39]
Cd^{2+}	*A. sphaerica***	5.5	25	111.1 mg g^{-1}	[35]
Cd^{2+}	*P. lilacinus*	6-7* 5-7**	30	36.46 mg g^{-1}* 41.99 mg g^{-1}	[38]
Cu^{2+}	*S. cerevisiae***	6.0	28	28.8 mg g^{-1}	[43]
Cu^{2+}	*Arthrobacter* ps-5**	5.0	28	169.15 mg g^{-1}	[44]
Cu^{2+}	*B. subtilis***	6.0	37	100.70 mg g^{-1}	[40]
Cu^{2+}	*A. niger*	5.3	30	25.3 mg g^{-1}	[30]
Cu^{2+}	*A. terreus***	6.0	50	15.24 mg g^{-1}	[19]
Cu^{2+}	*A. niger*	4.0-6.0	30	23.8 mg g^{-1}	[36]
Cu^{2+}	*S. cerevisiae*	5.5	22	29.9 mg g^{-1}	[37]
Cu^{2+} Cd^{2+}	*G. toebii (*G1) ** *G. thermoleovorans (*G2*)* **	Cd=6.0 Cu=4.0	Cd=70 Cu=60	G1: Cd=29.2 mg g^{-1} Cu=48.5 mg g^{-1} G2: Cd=38.8 mg g^{-1} Cu=41.5 mg g^{-1}	[33]
Cu^{2+} Cd^{2+}	*P. chrysosporium***	6.0	25	Cd= 27.79 mg g^{-1} Cu= 26.55 mg g^{-1}	[42]
Cu^{2+} Cd^{2+}	*B. bassiana*	6-8	30	Cu: 4.54 mg g^{-1} Cd: 4.5 mg g^{-1} (initial conc: 30 mg L^{-1})	[29]
Cu^{2+} Cd^{2+}	*Aspergillus* spp. 2 *Aspergillus* spp. 1	Cu: 4.0 Cd: 6.0	25	Cu: LB=5.36 DB=18.6 mg g^{-1} Cd: LB=0.19 DB=0.17 mg g^{-1}	In this study

*LB: Living Biomass, **DB: Dead Biomass



(a)



(b)

Figure 1. Isotherms for copper. (a) Freundlich and Langmuir isotherms for living fungus. (b) Langmuir isotherm for dead fungus

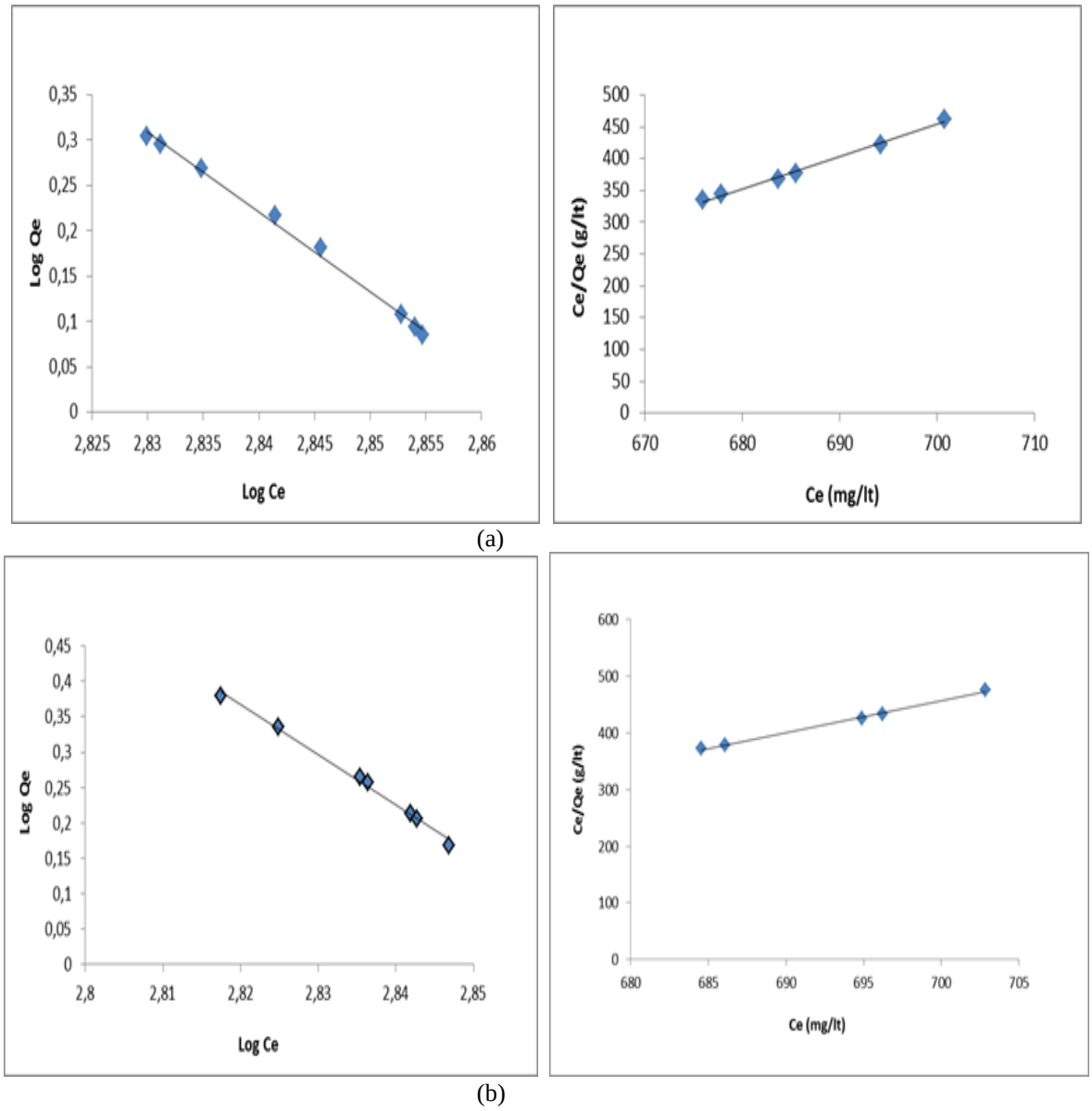


Figure 2. Freundlich and Langmuir isotherms for cadmium. (a) Bioaccumulation by living fungus. (b) Biosorption by dead fungus.

Table 3. Langmuir and Freundlich parameters for copper and cadmium biosorptions by *Aspergillus* spp.

		Langmuir			Freundlich		
		q_m (mg g^{-1})	K_L	R^2	1/n	K_f	R^2
Copper	Living biomass	5.3676	-0.1143	0.9993	-0.1796	13.231	0.9927
	Dead biomass	18.661	-0.0031	0.9446	-	-	-
Cadmium	Living biomass	0.1977	-0.00164	0.994	-8.8058	$1.694x10^{25}$	0.9955
	Dead biomass	0.1772	-0.00162	0.995	-7.0902	$2.301x10^{20}$	0.9939

A study about removal of Cu^{2+} by *Aspergillus niger* had found the biosorption capacity was 25.3 mg g^{-1} [30]. Acidophilic, heterotrophic, gram-negative *Acidiphilium symbioticum* H8 had studied for establish the mechanism of Cd^{2+} ion sorption. The sorption capacity was 248.62 mg cadmium per gram biomass at pH 6.0 and the process had explained by Langmuir–Freundlich dual isotherm model [34]. The study [35] had presented the cadmium biosorption from aqueous solution by the blue green algae *Anabaena sphaerica*. To describe the biosorption isotherms, Freundlich, Langmuir, and Dubinin–Radushkevich (D–R) models had been applied and the experimental data had fitted to Freundlich and Langmuir

isotherms. The mean free energy value had been calculated from the D–R plot was 14.3 kJ mol^{-1}, had indicated that the biosorption type was chemisorption.

The biosorption of Cu^{2+} from aqueous solutions by *Aspergillus niger* which had treated by rice straw had investigated and the biosorption capacity was 23.8 mg g^{-1} [36]. The study [37] on copper and lead removal from aqueous solutions by using *Saccharomyces cerevisiae* had resulted in the way that the maximum biosorption capacity of Pb^{2+} had better than Cu^{2+} (72.5 mg g^{-1} and 29.9 mg g^{-1}, respectively). Cadmium biosorption of the high cadmium resistant fungus *Paecilomyces lilacinus* had investigated for living and dead biomass. Adsorpted cadmium amounts had determined was 36.43 mg g^{-1} and 41.99 mg g^{-1} for living and dead biomass respectively [38].

Biosorption capacity of immobilized *Bacillus subtilis* beads (IBSB) for cadmium ions had studied and the equilibrium biosorption capacity had found to be 251.91 and 188 mg g^{-1} at temperatures of 45 °C and 25 °C, respectively. The results had showed that the biosorption capacity increased with increasing temperature [39].

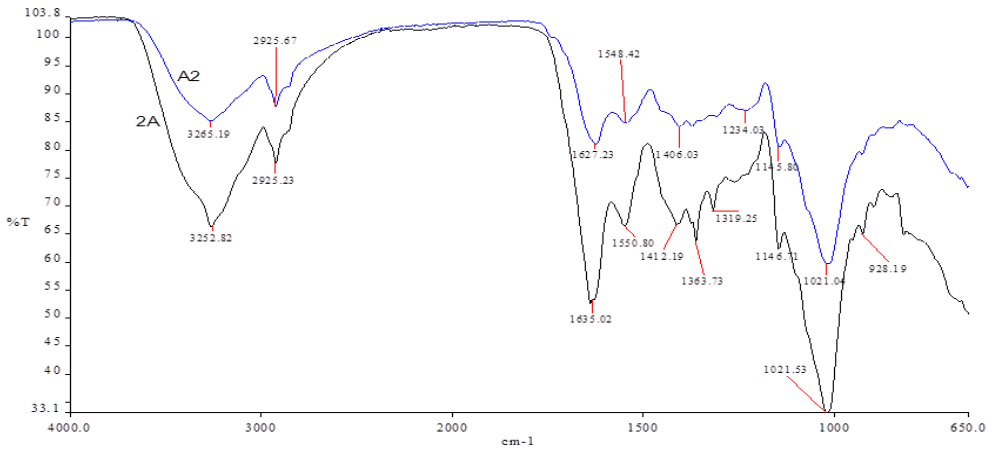
Immobilized *Bacillus subtilis* chitosan beads (BICB) had used for removal of copper ions from aqueous solution [40]. The equilibrium studies had described by four isotherm models as Langmuir, Freundlich, Temkin, Dubinin-Radushkevich and the Langmuir isotherm model had been the best description of the copper adsorption mechanism. The maximum adsorption capacity of copper had found 100.70 mg g^{-1}.

Micrococcus luteus DE2008 has the ability to absorb lead and copper. The microorganism had showed a greater tolerance for lead than copper. The biosorption capacities had found 408 mg g^{-1} for copper and 1965 mg g^{-1} for lead [41]. The biosorption of heavy metals (Cd(II), Pb(II) and Cu(II)) from artificial wastewaters onto the dry fungal biomass of *Phanerochaete chryosporium* had studied. The Langmuir isotherm model had fitted well for the adsorption equilibrium data and the biosorption capacities had found to be 23.04 mg g^{-1}, 69.77 mg g^{-1} and 20.23 mg g^{-1} for cadmium, lead and copper, respectively [42]. According to study of on copper biosorption by a wild type (BY4741) and two engineered *S. Cerevisiae* biomass (Rim101Δ, Och1Δ) [43], maximum biosorption capacities of copper had found to be 28.8, 8.0, 7.5 mg g^{-1} at pH=6 for wild type, Rim101Δ and Och1Δ respectively. The bioaccumulation capacities of these three strains had not found significantly different.

3.6. FTIR analyses

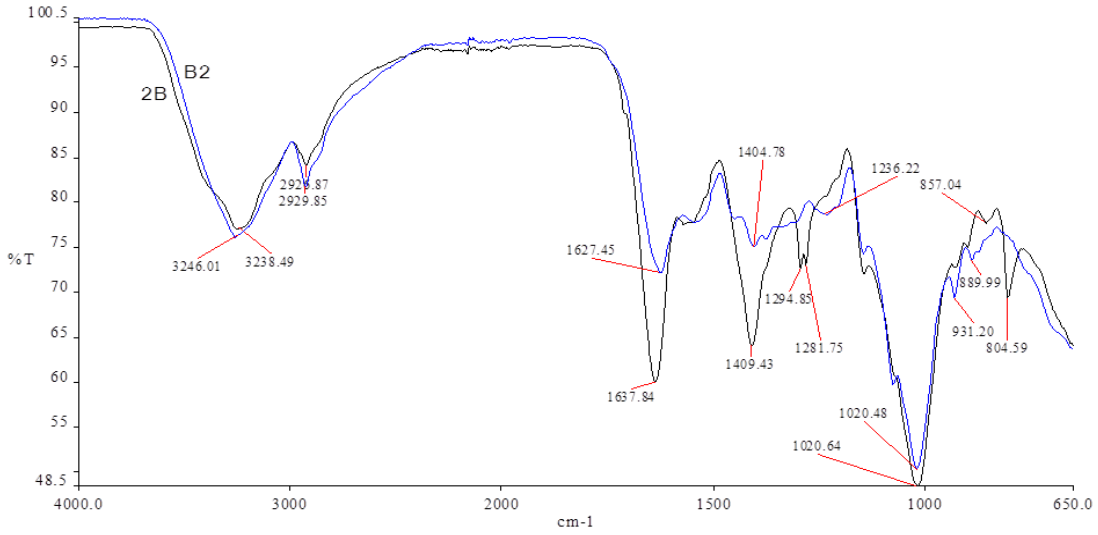
FTIR analyses were carried out for determining the metal binding sites on fungal cell surface. As seen on Fig.3 the copper binding sites on living fungus cell surface were isopropyl group (1380-1360 cm^{-1}), sulphones (1335-1295 cm^{-1}), vinyl compounds (950-900 cm^{-1}). Upon dead fungus cell surface, the copper were binding on primer amides (1420-1400 cm^{-1}), pridin N-oxydes (1300-1200 cm^{-1}), ethers (1240-1070 cm^{-1}), vinyl compounds (950-900 cm^{-1}), vinylidenes (900-865 cm^{-1}), triazine (820-800 cm^{-1}) groups. Besides, the cadmium binding sites on living fungus cell surface were esters (1750-1740 cm^{-1}), primer amides (1420-1400 cm^{-1}), alkyl aril esters (1285-1240 cm^{-1}). Upon dead fungus cell surface, the cadmium were binding on carbonyl compounds (1870-1650 cm^{-1}), aliphatic nitro compounds (1575-1545 cm^{-1}), primer amides (1420-1400 cm^{-1}), 1.2.4-trisubst benzenes (890-805 cm^{-1}) (Fig.4).

The biosorption behaviors for copper by a novel extracted exopolysaccharide (EPS) from *Arthrobacter* ps-5 had studied and the biosorption capacity of copper had found 169.15 mg g^{-1}. Interactions between metal ions and O–H, C=O, C–O–C, C=O–C groups of the EPS had determined by FTIR [44]. The FTIR spectra of *Saccharomyces cerevisiae* had had several functional groups as carboxyl, hydroxyl, amino and carbonyl groups on the cell wall [45]. Results of the biosorption of Cu^{+2} ions by *Saccharomyces cerevisiae* verified that sorption was a combination of intracellular metal accumulation and metabolic-independent surface phenomenon [37].



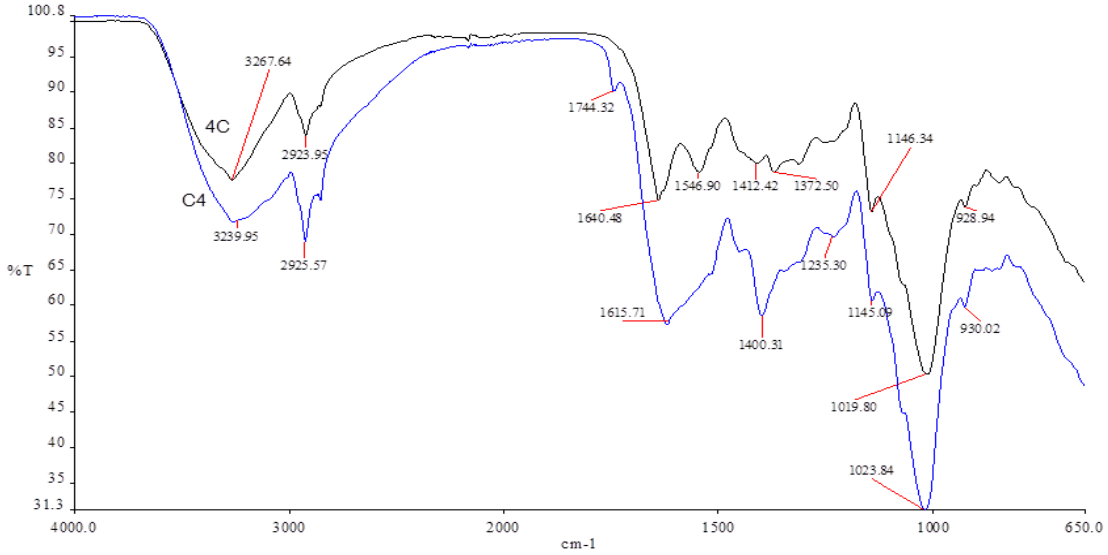
(a)

Figure 3. FTIR spectra of copper. (a) Bioaccumulation by living fungus. Blue lines in spectra presented control groups; black lines have indicated the metal-treated samples

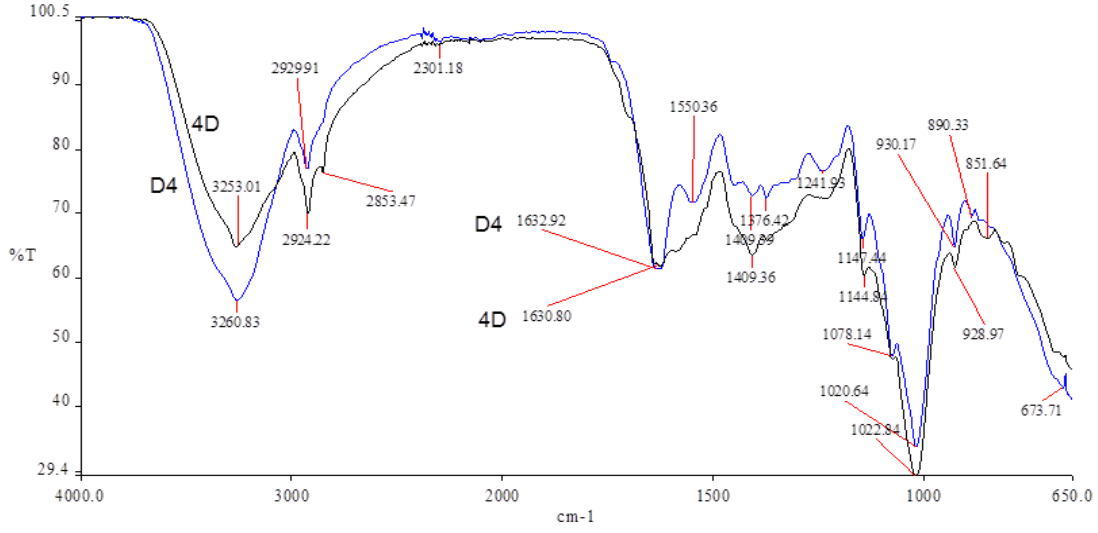


(b)

Figure 3. FTIR spectra of copper. (b) Biosorption by dead fungus. Blue lines in spectra presented control groups; black lines have indicated the metal-treated samples



(a)



(b)

Figure 4. FTIR spectra of cadmium. (a) Bioaccumulation by living fungus. (b) Biosorption by dead fungus. Blue lines in spectra presented control groups; black lines have indicated the metal-treated samples

3.7. SEM micrographs and EDX spectra

SEM micrographs (Fig.5) have shown that the metal absorbed filaments of dead fungus were more apparent than living fungus for Cu^{2+}. The exact opposite situation were observed for Cd^{2+} absorbed fungus filaments. Metal filled living fungus filaments were clearer than dead fungus filaments of living and dead biomass.

EDX determinate removal of cadmium were 97% and 95%; copper removal ratios were 90% and 88% for living and dead biomass respectively. EDX spectra have shown by Fig.6.

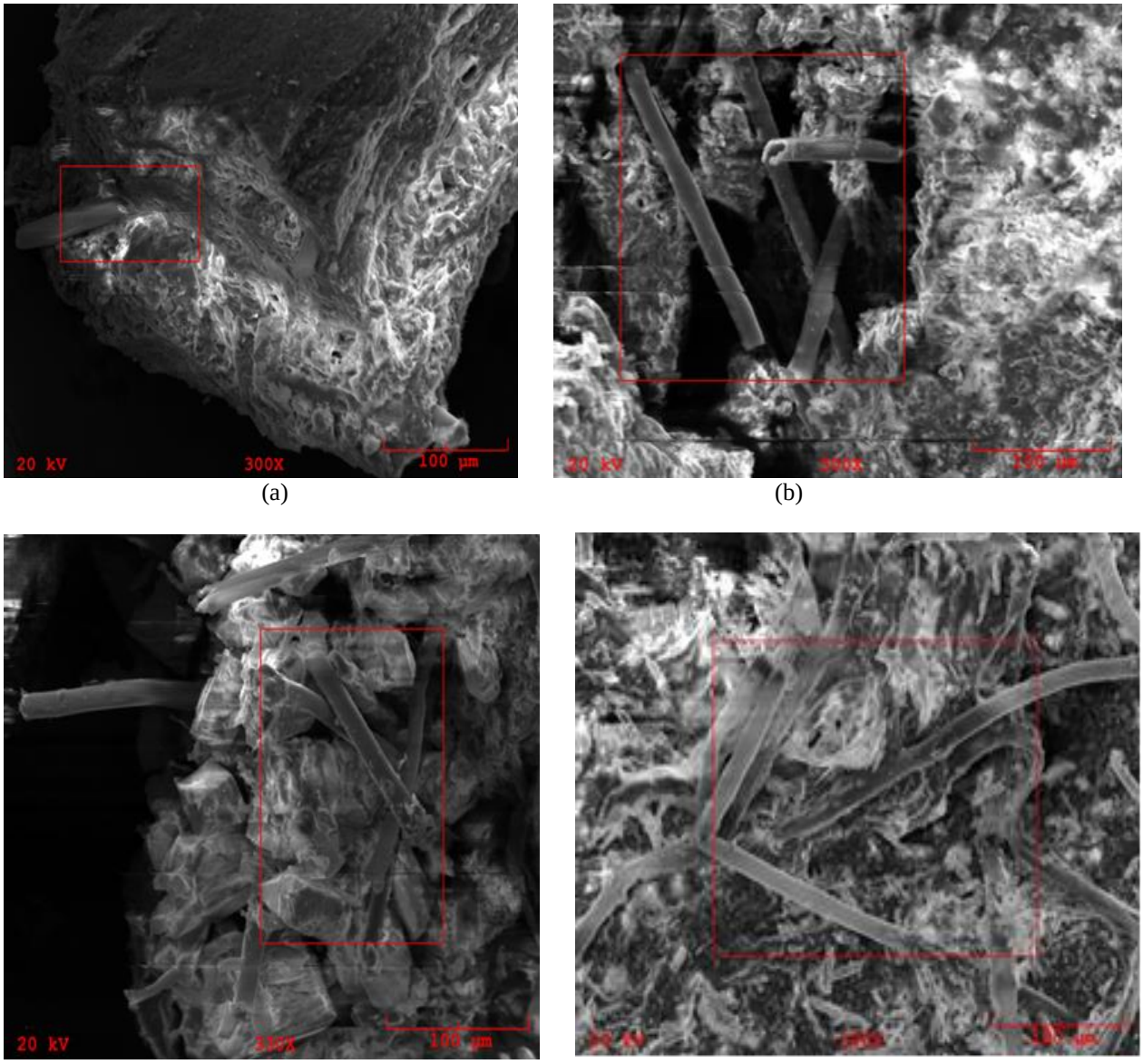
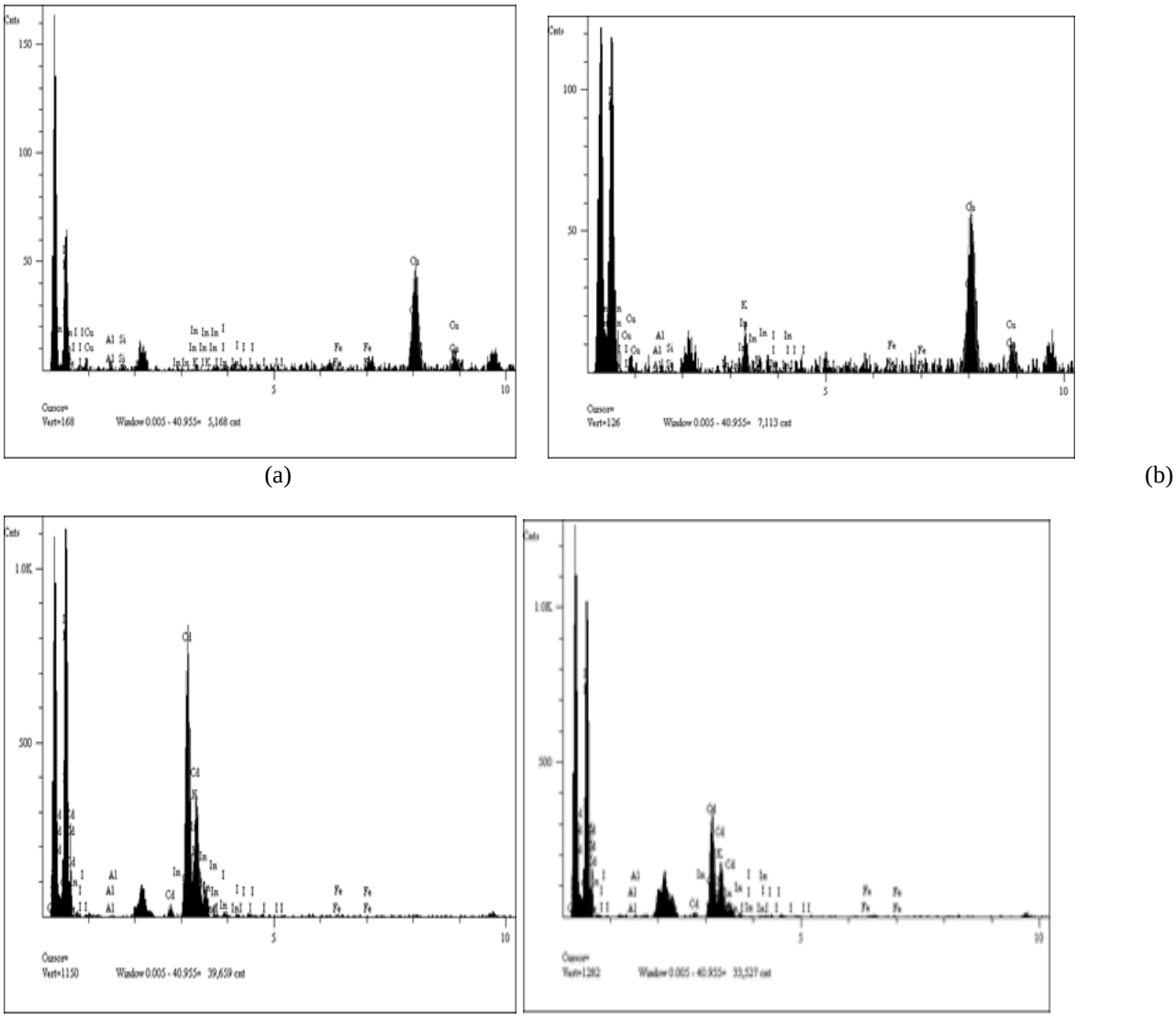


Figure 5. SEM micrographs of copper. (a) Bioaccumulation by living fungus. (b) Biosorption by dead fungus. SEM micrographs of cadmium. (c) Bioaccumulation by living fungus. (d) Biosorption by dead fungus.



(a) (b)

(c) (d)

Figure 6**.** EDX spectra of copper. (a) Bioaccumulation by living fungus. (b) Biosorption by dead fungus. EDX spectra of cadmium (c) Bioaccumulation by living fungus. (d) Biosorption by dead fungus.

4. Conclusions and discussion

- Ceramic waste sludge revealed ten fungi strains in *Aspergillus* sp. (2 strains), *Mucor* sp., *Penicillium* sp. (5 strains), *Rhizopus* sp. and *Trichoderma* sp.). Regard to MIC values *Aspergillus* sp. strains were chosen for copper and cadmium bio removal experiments.
- The removal efficiencies for cadmium and copper with two *Aspergillus* strains were found to be 90-95% and 85-90%, respectively.
- The sorption capacities of live and dead fungi for copper were 5.3676 mg g^{-1}, 18.661 mg g^{-1} and for cadmium were 0.1977 mg g^{-1}, 0.1772 mg g^{-1} respectively. Negative values were obtained in Langmuir and Freundlich isotherm constants for copper and cadmium biosorption processes. According to that results, Langmuir and Freundlich isotherm models did not fit the experimental data of biosorption and bioaccumulation. The studies may continue by working higher initial concentration or using other isotherm models.
- FTIR analysis have shown that copper ions bound to vinyl compounds (950-900 cm^{1}) and cadmium ions bound to primer amides (1420-1400 cm^{-1}) mainly.
- SEM micrographs were shown that the metal absorbed filaments of living fungus and dead fungus had different appearance for Cu^{2+} and Cd^{2+}. EDX analyses have proven that *Aspergillus* spp. was a good biosorbent for cadmium and copper.
- As a result, the initial metal concentration was found to be significant and effective for biosorption capacity.

As a result, besides physical or chemical adsorption methods of heavy metals, it is possible to obtain high yields in removal by biosorption method. In experimental studies it was observed that initial concentration and fungus strain were effective in biosorption yield. If this work is to be continued; biosorption experiments can be performed with different heavy metals or different concentrations of the same metals. It is also possible to determine the optimum conditions for biosorption studies that can be performed by changing parameters such as pH, temperature, agitation speed, contact duration. Biosorption experiments with metal-resistant bacteria can also be performed.

Acknowledgements

This work was financially supported by the Unit of The Scientific Research Projects of Anadolu University under grant no. 1203F055.

References

[1] Ziemacki, G., Viviano, G. & Merli F. (1989). Heavy metals: Sources and environmental presence. *Annali dell'Istituto Superiore di Sanità*, 25(3), 531-535.
[2] Chandra, R., Bharagava, R.N., Yadav, S. & Mohan, D. (2009). Accumulation and distribution of toxic metals in wheat (*Triticum aestivum L.*) and indian mustard (*Brassica campestris L.*) irrigated with distillery and tannery effluents. *Journal of Hazardous Materials*, 162 (2-3), 1514-1521.
[3] Akpor, O. B. & Muchie, M. (2010). Remediation of heavy metals in drinking water and wastewater treatment systems: processes and applications. *International Journal of Physical Science*, 5(12), 1807-1817.
[4] www.esdat.net national primary drinking water regulations (Accessed: September 2018)
[5] Fomina, M. & Gadd, G. M. (2014). Biosorption: current perspectives on concept, definition and application. *Bioresource Technology*, 160, 3-14.
[6] Bilal, M., Shah, J. A., Ashfaq, T., Gardazi, S. M. H., Tahir, A. A., Pervez, A., Haroon, H. & Mahmood, Q. (2013). Waste biomass adsorbents for copper removal from industrial wastewater-A review. *Journal of Hazardous Materials*, 263, 322-333.
[7] Chojnacka, K. (2010). Biosorption and bioaccumulation – the prospects for practical applications, *Environment International*, 36, 299-307.
[8] Turhan, S., Arikan, I. H., Demirel, H. & Gungor, N. (2011) Radiometric analysis of raw materials and products in the turkish ceramics industry. *Radiation Physics and Chemistry*, 80 (5), 620-625.
[9] Monfort, E., Mezquita, A., Vaquer, E., Celades, I., Sanfelix, V. & Escrig, A. (2014). Ceramic manufacturing processes: energy, environmental, and occupational health issues. *Comprehensive Materials Processing*, 8, 71-102.
[10] Dincer, A. R. & Kargi, F. (2000). Seramik endüstrisi atıksularının özellikleri ve biyolojik arıtılabilirliği, Çevre Bilim&Teknoloji 1(1), 24-28.
[11] Sepehr, M.N., Zarrabi, M. & Amrane, A. (2011). Removal of Cr(III) from model solutions by isolated *Aspergillus niger* and *Aspergillus oryzae* living microorganisms: Equilibrium and kinetic studies, *Journal of Taiwan Institute of Chemical Engineers*, 43(3), 420-427.
[12] https://www.epa.gov/sites/production/files/2015-12/documents/3051a.pdf (Accessed: March 2017).
[13] Piotrowska-Seget, Z., Cycon, M. & Kozdroj, J. (2005). Metal-tolerant bacteria occurring in heavily polluted soil and mine spoil. *Applied Soil Ecology*, 28(3), 237-246.
[14] Klich, M.A. (2002). İdentification of common *Aspergillus* species. Centraalbureau voor Schimmelcultures. Ulrecht, Netherlands.
[15] Dugan, F.M. (2006). The identification of fungi: an illustrated introduction with keys, glossary and guide to literature. American Phytopathological Society. St. Paul, Minnesota.
[16] Watanabe, T. (2010). Pictorial atlas of soil and seed fungi: morphologies of cultured fungi and key to species. CRC Press/Taylor & Francis. Boca Raton.
[17] Flannigan, B., Samson, R. A. & Miller, J. D. (2011). Microorganisms in home and indoor work environments: diversity, health impacts, investigation and control. Taylor&Francis. Boca Raton, Florida.
[18] Zafar, S., Aqil, F. & Ahmad, I. (2007). Metal tolerance and biosorption potential of filamentous fungi isolated from metal contaminated agricultural soil. *Bioresource Technology*, 98(13), 2557-2561.
[19] Cerino-Cordova, F. J., Garcia-Leon, A. M., Soto-Regalado, E., Sanchez-Gonzalez, M. N., Lozano Ramirez, T., Garcia-Avalos, B. C. & Loredo-Medrano, J. A. (2012). Experimental design for the optimization of copper biosorption from aqueous solution by *Aspergillus terreus*. *Journal of Environmental Management*, 95, 77-82.
[20] Subbaiah, M. V., Yuvaraja, G., Vijaya, Y. & Krishnaiah, A. (2011). Equilibrium, kinetic and thermodynamic studies on biosorption of Pb(II) and Cd(II) from aqueous solution by fungus (*Trametes versicolor*) biomass. J*ournal of the Taiwan Institute Chemical Engineers*, 42(6), 965-971.
[21] Tay, T. & Keçili, R. (2016). Removal of Acid Blue 294 (AB294) dye from aqueous solutions by using lichen Umbilicaria decussata biomass, *Biological Diversity and Conservation*, 9/3, 189-197.

[22]https://www.epa.gov/rcra/resource-conservation-and-recovery-act-rcra-regulations#haz (Accessed: September 2018).
[23]https://www.brighthubengineering.com/manufacturing-technology/56841-what-are ceramic-materials-and-their-uses/ (Accessed: October 2018).
[24] Ujile, A. A. & Joel, O. F. (2013). Adsorption process of iron(III) from borehole water on activated carbon from nigerian bamboo. *International Journal of Engineering Science and Technology*, 5(6), 1321-1331.
[25] Karthikeyan, G., Muthulakshmi, A. N. & Anbalagan, K. (2005). Adsorption studies of iron (III) on chitin, *Indian Academy of Sciences*, 117(6), 663-672.
[26] Dange, S.S. & Dhoble, R.M. (2017). Iron Removal by Adsorption-A review**,** *International Journal of Science Technology* & *Engineering*, 3 (9), 603-606.
[27] Hoyland, V. W., Knocke, W. R., Falkinham, J. O. 3rd., Pruden, A. & Singh, G. (2014). Effect of drinking water treatment process parameters on biological removal of manganese from surface water. *Water Research*, 66, 31-39.
[28] Mishra, A. & Malik, A. (2012). Simultaneous bioaccumulation of multiple metal from electro plating effluent using *Aspergillus lentulus*. *Water Research*, 46, 4991-4998.
[29] Gola, D., Dey, P., Bhattacharya, A., Mishra, A., Malik ,A., Namburath, M. & Ahammad, S.Z. (2016). Multiple heavy metal removal using an entomopathogenic fungi *Beauveria bassiana*. *Bioresource Technology*, 218, 388-396.
[30] Wang, J. Y. & Cui, C. (2017). Characterization of the biosorption properties of dormant spores of *Aspergillus niger*: A potential breakthrough agent for removing Cu^{2+} from contaminated water. *RSC Advances*, 7, 14069–14077.
[31] Chakraborty, S., Mukherjee, A., Khuda-Bukhsh, A.R. & Das, T.K. (2014). Cadmium induced oxidative stress tolerance in cadmium resistant *Aspergillus foetidus*: Its possible role in cadmium bioremediation. *Ecotoxicology and Environmental Safety*, 106, 46-53.
[32] Li, C., Jiang, W., Ma, N., Zhu, Y., Dong, X., Wang, D., Meng, X. & Xu, Y. (2014). Bioaccumulation of cadmium by growing *Zygosaccharomyces rouxii* and *Saccharomyces cerevisiae*. *Bioresource Technology*, 155, 116-121.
[33] Ozdemir, S., Kilinc, E., Poli, A., Nicolaus, B. & Guven, K. (2009). Biosorption of Cd, Cu, Ni, Mn and Zn from aqueous solutions by thermophilic bacteria, *Geobacillus toebii sub. sp. decanicus* and *Geobacillus thermoleovorans sub. sp. stromboliensis*: equilibrium, kinetic and thermodynamic studies. *Chemical Engineering Journal*, 152(1), 195-206.
[34] Chakravarty, R. & Banerjee, P.C. (2012). Mechanism of cadmium binding on the cell wall of an acidophilic bacterium. *Bioresource Technology*, 108, 176-183.
[35] Abdel-Aty, A. M., Ammar, N. S., Abdel Ghafar, H. H. & Ali, R. K. (2013). Biosorption of cadmium and lead from aqueous solutions by fresh water alga *Anabaena sphaerica* biomass. *Journal of Advanced Research*, 4(4), 367-374.
[36] Wang, J. Y., Cui, H., Cui, C. W. & Xing, D. F. (2016). Biosorption of copper(II) from aqueous solutions by *Aspergillus niger* treated rice straw. *Ecological Engineering*, 95, 793-799.
[37] Amirnia, S., Ray, M. B. and Margaritis, A. (2015). Heavy metals removal from aqueous solutions using *saccharomyces cerevisiae* in a novel continuous bioreactor-biosorption system. *Chemical Engineering Journal*, 264, 863-872.
[38] Zeng, X., Chai, L., Tang, J., Liu, X. & Yang, Z. (2013). Taxonomy characterization and cadmium biosorption of fungus strain. *Transactions of Nonferrous Metals Society of China*, 23, 2759-2765.
[39] Ahmad, M. F., Haydar, S., Bhatti, A. A. & Bari, A. J. (2014). Application of artificial neural network for the prediction of biosorption capacity of immobilized *Bacillus subtilis* for the removal of cadmium ions from aqueous solution. *Biochemical Engineering Journal*, 84, 83-90.
[40] Liu, Y., Liao, T., He, Z., Li, T., Wang, H., Hu, X., Guo, Y. & He, Y. (2013). Biosorption of copper (II) from aqueous solution by *Bacillus subtilis* Cells immobilized into chitosan beads. *Transactions of Nonferrous Metals Society of China*, 23(6), 1804-1814.
[41] Puyen, Z. M., Villagrasa, E., Maldonado, J., Diestra, E., Esteve, I. & Sole, A. (2012). Biosorption of lead and copper by heavy-metal tolerant *Micrococcus luteus* DE2008. *Bioresource Technology*, 126, 233-237.
[42] Say, R., Denizli, A. & Arica, M. Y. (2001). Biosorption of cadmium(II), lead(II) and copper(II) with the filamentous fungus *Phanerochaete chrysosporium*. *Bioresource Technology*, 76, 67-70.
[43] Di Caprio, F., Altimari, P., Uccelletti, D. & Pagnanelli, F. (2014). Mechanistic modelling of copper biosorption by wild type and engineered *Saccharomyces cerevisiae* biomasses, *Chemical Engineering Journal*, 244, 561-568.
[44] Shuhong, Y., Meiping, Z., Hong, Y., Han, W., Shan, X., Yan, L. & Jihui, W. (2014). Biosorption of Cu^{2+}, Pb^{2+} and Cr^{6+} by a novel exopolysaccharide from *Arthrobacter* ps-5. *Carbohydrate Polymers*, 101(1), 50-56.
[45] Wang, J. (2002). Biosorption of copper(II) by chemically modified biomass of *Saccharomyces cerevisiae*, *Process Biochemistry*, 37(8), 847-850.

(Received for publication 28 January 2019; The date of publication 15 December 2019)