BIOSORPTION OF HEAVY METALS BY USING PRETREATED BIOMASS OF METAL-TOLERANT FUNGI

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Abstract

Soil samples were collected from selected area of Pathein Township during June to July, 2015. Fourteen different soil fungi were isolated by using physical treatment dilution method and soil plating method. All isolates were initially grown on PGA medium and selected heavy metals such as Cu^{2+}, Cr^{6+} and Ni^{2+} tolerance activities were checked by plate diffusion method. In plate diffusion method, results of zone formation indicate the ability of the isolates as heavy metal-resistant or sensitive. Based on their zone of inhibition, the maximum tolerance levels of isolates were chosen. Four different fungi (OMA-4, OMA-9, OMA-11 and OMA-14) were found to possess the metal tolerance potential in terms of minimum inhibitory concentration (MIC). Moreover, functional group and elemental analysis of active surface of fungi were characterized by FT IR and EDXRF analyses. The metal-tolerant fungi were carried out to investigate the production of biomass by time course of incubation, effect of pH, effect of carbon source consumption and effect of nitrogen source consumption. The observed conditions were applied for the biosorption process by effect of solution pH, biomass dosage, metal ion concentration and contact time. Experimental results revealed that the isolated soil fungi have shown a high level of resistance to tested metals and which makes them attractive potential for their ability to remove metal ions.

Keywords: PGA, plate diffusion method, MIC, metal-tolerant, biosorption

Introduction

The use of natural materials in the removal of heavy metals is becoming a concern in all countries. Heavy metals have been excessively released into the environment due to rapid industrialization and have created a major global concern. Heavy metal ions have effects on all forms of life. Heavy metal pollution is one of the most important environmental problems today because of their toxicity, bio-accumulation tendency, threat to human life and environment. Heavy metals are present in nature and industrial waste water, so the presence of heavy metals in surface and ground water pose a

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contamination problem. Large number of industries can produce and discharge wastes containing different heavy metals into the environment.

The main source of heavy metal pollution are metal plating, mining, smelting, battery manufacturing, tanneries, pulp factories and etc. Removal of heavy metals from industrial wastewater is of primary importance. Cadmium, zinc, copper, nickel, lead, mercury and chromium are often detected in industrial wastewaters. Biosorption is the removal of materials (compounds, metal ions etc.) by inactive, non-living biomass due to high attractive forces between the two. Living as well as dead (metabolically inactive) biological materials have been sought to remove metal ions. Microorganism like bacteria, fungi, yeast, and algae from their natural habitats are excellent sources of biosorbent. These biosorbents possess metal-sequestering property and can be used to decrease the concentration of heavy metal ions in solution from ppm to ppb level (Kapoor, 1998).

The aim of this study was to determine the biosorption of heavy metals by using pretreated biomass of metal-tolerant fungi.

Materials and Methods

Isolation of Soil Fungi

Sampling

Seven different kinds of soil samples were collected within the studied area of Pathein Township during June to July, 2015. Soil texture and pH were analyzed at the Department of Agriculture (Land Use), Yangon. The collected soil sample was air-dried at room temperature. It was ground and sieved with 2mm screen.

Media used for the isolation of soil fungi

1. LCA Medium (Ando, 2004)

Glucose	2.0 g
Sucrose	2.0 g
K_2HPO_4	1.0 g
$MgSO_4.7H_2O$	0.5 g
KNO_3	1.0 g
KCl	0.5 g
Agar	18 g
DW	1000 mL
pH	6.5

2. PGA Medium (Ando, 2004)

Potato	200 g
Glucose	20 g
Agar	18 g
Yeast Extract	1.0 g
DW	1000 mL
pH	6.5

(After autoclaving chloramphenicol 0.03 g was added to the medium)

Physical treatment dilution method

The soil sample was placed in hot air oven at 120 ^{0}C for 1 h. The dried soil sample was diluted with sterile water. The final test tubes of dilution series were cultured on low carbon agar (LCA) medium and incubated for 5 to 7 days. After incubation, pure colonies were obtained by cultured in potato glucose agar (PGA) medium (Phay and Yamamura, 2005).

Soil plating method

0.1 g of soil sample was put into empty petri dish. Nutrient agar (LCA medium) was poured into the petri dish and shaken gently to suspended soil particles in medium. The medium was cooled down to solidify.

Determination of the Selected Metal - Tolerant Fungi by Plate Diffusion Method

Metal ions (Cu^{2+}, Ni^{2+}, Cr^{6+}) in aqueous solutions were prepared at different concentrations of 10, 20, 30, 40, 50 mM. Each plate was spread with 72 h cultures of appropriate organisms. To each plate, 100 µL of the selected

metal salt solutions were added in each well of 10 mm in diameter and 4 mm in depth. Fungal plates were incubated at 30 °C for 72 h. After incubation, the zone of inhibition was observed (Hassen, 1998).

Isolates which showed significant growth and tolerance to high concentration of metal were ranked and best strains were selected.

Studies on Biomass Production (Cell Weight) of Metal-tolerant Fungi
Optimization of time course

Time course for biomass production was investigated in potato glucose liquid broth (PGB) medium, with initial pH 6.5 at room temperature. Representative time course of biomass production for selected fungi were incubated for 3 to 10 days. After incubation, fungal biomass was estimated gravimetrically by filtering the culture through a preweighed dry Whatmann No.1 filter paper. Mycelium was thoroughly washed with distilled water and dried in oven at 80 °C until constant weight was obtained.

Effect of pH

The production of biomass depends on the initial pH of the fermentation medium. The metal-tolerant fungi OMA-4, 9, 11 and 14 were incubated in a series of 100 mL of PGB medium at different pH values from 3 to 7 at room temperature. The pH of the medium was adjusted using 0.1 N HCl or 0.1 N NaOH.

Effect of carbon source consumption

In this experiment, potato glucose broth (PGB) medium was used as basal medium incorporated with soluble starch, sucrose, and rice bran were used as carbon source. Each of carbon sources were added in PGB medium at the concentration of 1.2 g per 100 mL and incubated for 7 days at room temperature. The experiment was compared to the results from the control (PGB) medium without carbon source addition.

Effect of nitrogen source consumption

The maximum biomass production was studied with respect to variation of nitrogen source such as yeast extract, peptone and $(NH_4)_2\ SO_4$.

Each of nitrogen sources were added in the PGB medium at a dosage of 0.8 g per 100 mL distilled water and incubated for 7 days at room temperature.

Preparation of the pretreated biomass

A liquid medium potato glucose broth (PGB) was prepared by 200 g of potato boiled in distilled water and the filtrate was mixed with 2 g of glucose and the volume made up to1000 mL of distilled water. It was sterilized by autoclaving (15 lb pressure) at 121 °C for 15 min. After autoclaving, 50 mL medium were inoculated by fungal mycelium from 3-5 days old culture and incubated at 25 °C for 7 days. Fungal cell mass was prepared by filtering the culture medium through filter paper.

Mycelium was thoroughly washed with deionized water and suspended in 0.5 N NaOH solution. The pretreated biomass was washed with deionized water until the pH of the solution was adjusted to neutral range. Biomass was autoclaved for 15 min at 121 °C and dried in an oven at 80 °C for 24 h. Dried biomass was ground and sieved. The prepared biomass powder was stored in air tight bottle for further biosorption experiment (Raja Rao, 2013).

Characterization of Biomass by FT IR and EDXRF Analysis

The prepared biomass powder were preliminary characterized by FT IR and EDXRF techniques (Salmann, 2010). The procedure was in accordance with recommended procedure as reported in FT IR and EDXRF instruments, Shimadzu, Japan.

Study on Biosorption of Metal-Tolerant Fungi

Effect of solution pH

Biomass powder (0.5 g) was added in the conical flask containing 50 mL of 20 mM concentration of metal solutions. The pH of metal solutions were varied from 3, 4, 5, 6, 7 to 8. The mixture was shaken at shaker and after 3 h contact time, the mixture was filtered. Then the residual colour of metal solutions were determined by UV-Vis spectrophotometer at maximum wavelengths of Cu^{2+}, Ni^{2+} and Cr^{6+} were 620 nm, 420 nm and 540 nm respectively.

Effect of adsorbent dosage

Accurate weight of biomass powder varying from 0.1 g to 0.5 g were mixed with 50 mL of metal salt solution in conical flask. The flask were placed on shaker with constant speed and left to equilibrate. After 3 h contact time, the solution was filtered and the absorbance of supernatant was measured by UV-Vis spectrophotometer.

Effect of metal ion concentration

Biomass powder (0.5 g) was added in the conical flask containing 50 mL of 10 mM metal solution. The mixture was shaken at shaker and after 3 h contact time, the mixture was filtered. Then the residual colour of metal solution was determined by UV-Vis spectrophotometer. Similarly, 20 mM, 30 mM, 40 mM and 50 mM of metal solution were also determined by above procedure.

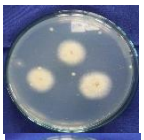
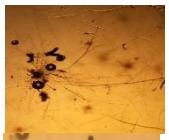
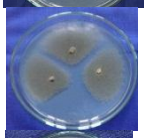
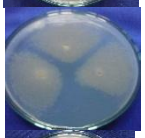
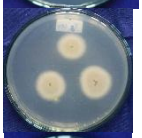
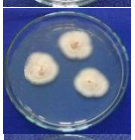
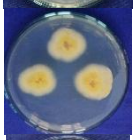
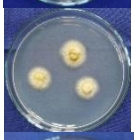
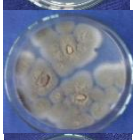
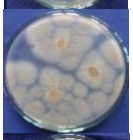
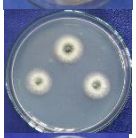
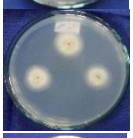
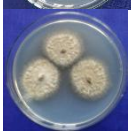
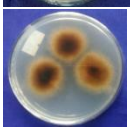
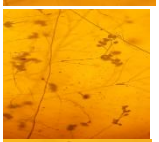
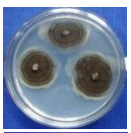
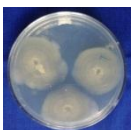
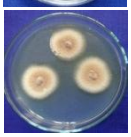
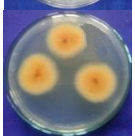
Effect of contact time

Accurate weight of 0.5 g biomass powder was placed in each 50 mL of metal solution in each flask. The flask were placed on shaker with constant speed for 1h. Then the sample was filtered and the absorbance of solution was determined by UV-Vis spectrophotometer. The contact time was varied for 2h, 4h, 6h, 8h and 10h. The residual colour of metal solution in terms of absorbance was determined by UV-Vis spectrophotometer.

Results and Discussion

Preliminary Study of Isolated Fungi

In the preliminary study, fourteen different fungi were isolated from soil samples and temporarily named as OMA-1 to OMA-14. All isolates of cultural morphology and photomicrographs are shown in Figure 1.

Temporary Name	Front View	Reverse View	Photomicrograph
OMA-1			
OMA-2			
OMA-3			
OMA-4			
OMA-5			
OMA-6			
OMA-7			
OMA-8			
OMA-9			
OMA-10			

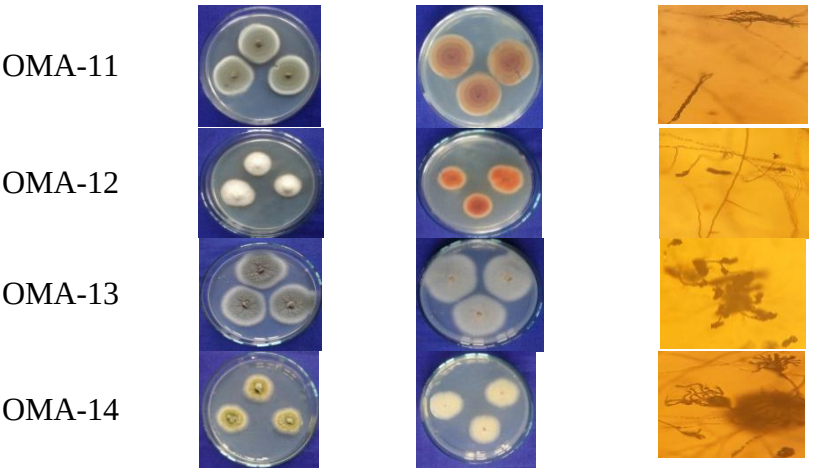


Figure 1: Colony characters and photomicrograph of fungi OMA-1 to OMA-14

Screening and Selection of Heavy Metal-Tolerant Fungi

The MIC of the highest metal-tolerant fungi are shown in Table 1. The minimum inhibitory concentration (MIC) is defined as the lowest concentration of metal that inhibit visible growth of test fungi. Results of zone formation indicated that the isolates were metal-resistant or sensitive. Metal- resistant isolates show no inhibition of growth at higher concentration of metals, whereas metal- sensitive isolates show inhibition of growth at higher concentration of metals (Hassen, 1998). Based on these concept, OMA-9 was resistant to Cu^{2+} metal ions, while OMA-11 was resistant to Cr^{6+} and OMA-14 was resistant to Ni^{2+} at MIC value 50 mM respectively (Figure 2).

Table 1 Minimum Inhibitory Concentration (MIC) of Tolerance Fungi

Metal ion	**MIC (mM)**			
	OMA-4	**OMA-9**	**OMA-11**	**OMA-14**
Cu^{2+}	20	50	20	10
Cr^{6+}	NG	NG	50	NG
Ni^{2+}	20	20	10	50

* NG- No Growth

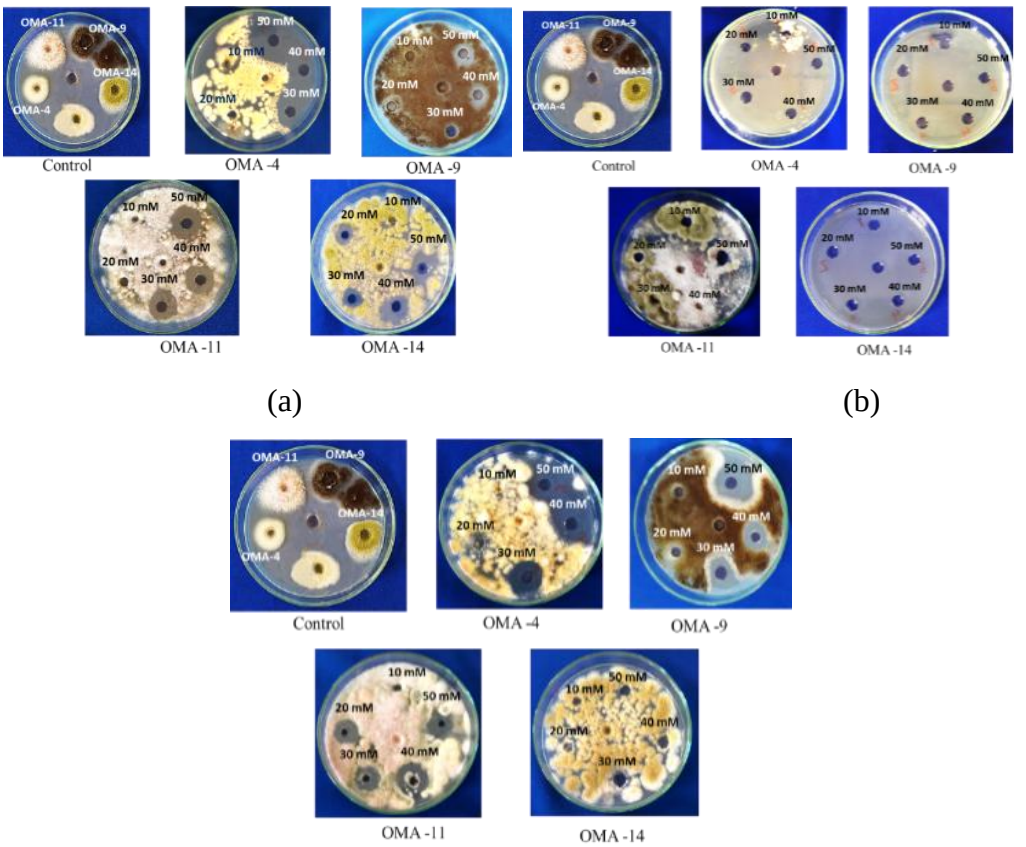


Figure 2: Fungal growth in different concentration of heavy metal (a) OMA-9 resistant to Cu^{2+} (b) OMA-11 resistant to Cr^{6+} (c) OMA-14 resistant to Ni^{2+}

Studies on Biomass Production Weight

In the study of time course (Table 2), OMA-9 grew much faster than others to reach the highest level of biomass after 6 days of incubation. Both OMA-4 and OMA-11 were observed to attain maximum growth for 7 days of incubation. OMA-14 was found to have shorter cultivation time (6 days) with lower biomass weight. pH is a significant effect on fungal biomass production. In this study, OMA-11 gave maximum biomass production at pH 6.5, OMA-9 and OMA-4 showed second best biomass production at pH 6.0 and pH 6.5 respectively. A significant less amount of biomass was obtained by OMA-14 at pH 5.5 (Table 3).

The carbon source and nitrogen source are key parameter of fungal cell growth. There was no obvious effect on biomass production by the addition of soluble starch and sucrose. OMA-11 gave the highest mycelium growth and OMA- 4 gave the lowest mycelium growth in rice bran (Table 4). Therefore, carbon source as rice bran was effective for biomass production. The increase in biomass weight was found by the addition of yeast extract and decrease by the addition of peptone and $(NH_4)_2$ SO_4. According to data, nitrogen source as yeast extract was best for growth of fungal biomass (Table 5).

Table 2: Effect of Time Course on Biomass Production Weight

Time (days)	Biomass Weight (g/100 mL)							
	OMA-4		OMA-9		OMA-11		OMA-14	
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
3	4.87	0.86	5.11	1.01	4.14	1.08	4.81	0.85
4	5.04	0.98	5.25	1.18	5.19	1.13	5.33	0.91
5	5.79	1.01	6.13	1.32	5.54	1.17	6.28	0.99
6	6.31	1.29	6.83	1.79	6.19	1.41	6.75	1.15
7	6.94	1.44	6.41	1.66	6.83	1.72	6.03	1.08
8	6.12	1.33	5.88	1.13	5.66	1.30	5.13	1.01
9	5.83	1.14	4.30	0.77	5.24	1.01	4.82	0.76
10	5.71	1.05	4.19	0.52	5.03	0.97	4.15	0.51

Table 3: Effect of pH on Biomass Production Weight

pH	Biomass Weight (g/100 mL)							
	OMA-4		OMA-9		OMA-11		OMA-14	
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
3.0	2.51	0.21	2.11	0.35	2.58	0.33	1.80	0.18
3.5	2.66	0.28	2.73	0.43	2.71	0.38	1.85	0.20
4.0	2.81	0.31	2.94	0.55	2.85	0.44	1.98	0.22
4.5	2.97	0.37	3.02	0.54	3.11	0.59	2.11	0.27
5.0	3.12	0.44	3.19	0.56	3.20	0.63	2.94	0.46
5.5	3.34	0.56	3.83	0.68	3.66	0.65	3.73	0.52
6.0	3.58	0.68	4.14	0.74	3.75	0.75	2.89	0.06
6.5	3.84	0.70	4.07	0.65	4.19	0.88	2.33	0.35
7.0	2.88	0.51	2.55	0.42	2.32	0.56	1.23	0.15

Table 4: Effect of Carbon Source on Biomass Production Weight

Carbon Source	Biomass Weight (g/100 mL)							
	OMA-4		OMA-9		OMA-11		OMA-14	
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
PGB (Control)	6.48	1.42	6.62	1.80	8.44	1.83	6.30	1.05
Rice Bran	9.18	1.55	7.04	1.97	10.93	2.15	8.68	1.69
Soluble Starch	5.71	1.23	5.63	1.29	6.83	1.71	4.69	1.29
Sucrose	4.72	1.05	5.42	1.24	5.81	1.13	3.68	1.03

Table 5: Effect of Nitrogen Source on Biomass Production Weight

Nitrogen Source	Biomass Weight (g/100 mL)							
	OMA-4		OMA-9		OMA-11		OMA-14	
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
PGB (Control)	6.48	1.42	6.62	1.80	6.88	1.83	6.30	1.05
Yeast Extract	8.66	1.56	8.75	1.88	9.75	1.92	13.06	1.73
Peptone	5.37	1.24	6.93	1.39	8.56	1.50	6.67	1.35
Ammonium Sulphate	3.48	1.07	3.69	1.09	5.56	1.12	3.61	1.17

Characterization of Metal-Tolerant Fungi by FT IR Analysis

Biosorption of the metals ion on the biomass highly depends on the functional groups on the active sites of biomass. Fungi offer a wide range of chemical groups that can attract and sequester the metals in biomass. Cell walls are composed of structural polysaccharides, proteins and lipids that offer metal binding functional groups (Veglio, 1997).To understand the biosorption mechanism of metal ions on to biomass, FT IR spectra of tolerant fungi were analyzed. FT IR spectral showed that, the peaks at 3417 cm^{-1}, 3385 cm^{-1} are due to the O-H and N-H stretching. The peaks at 2931 cm^{-1}, 2854 cm^{-1} are associated with the stretching vibration of C-H bonds of methyl and methylene group. The peaks at 1649 cm^{-1}, 1558 cm^{-1} are corresponding to the -C = C stretching which might be due to the presence of aromatic group. The bands observed at 1377 cm^{-1}, 1416 cm^{-1} are C-H bending and intense peaks at 1078 cm^{-1}, 1149 cm^{-1} are corresponding to the C-O stretching of alcohol or carboxylic group (Table 6). The results showed that the active surface of the biomass may include amine, carboxyl and aromatic groups, which are attributed to the complexation or coordination of the metal ions on to the biomass.

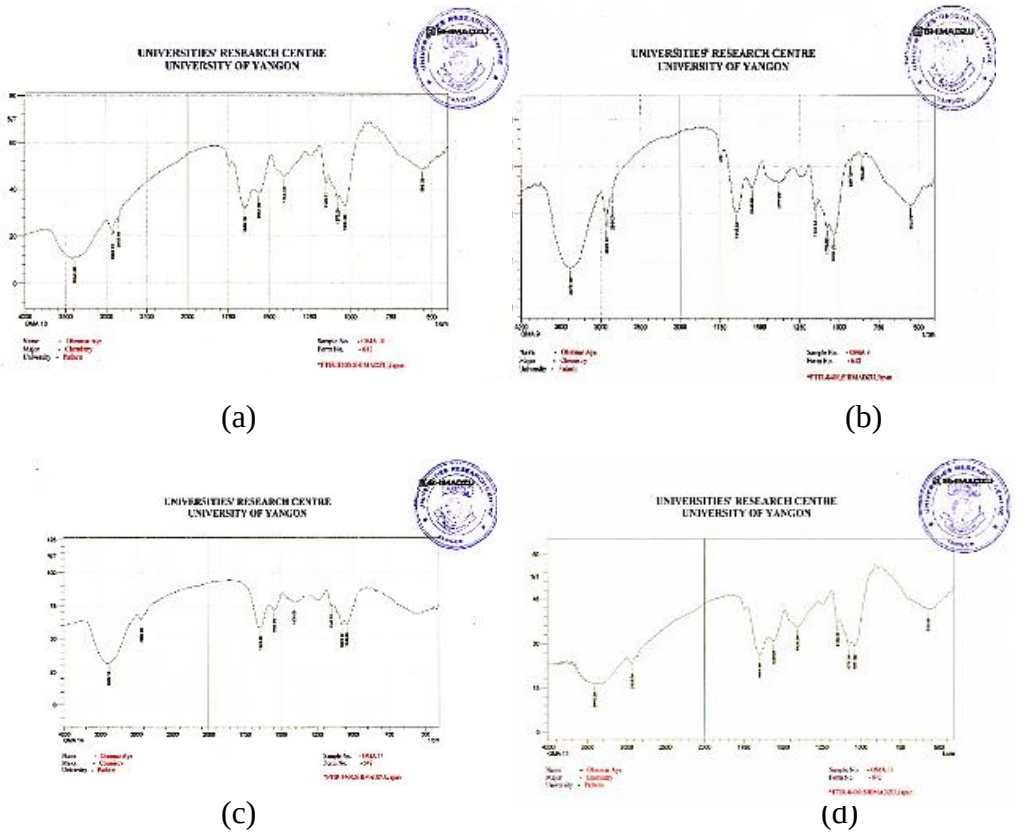


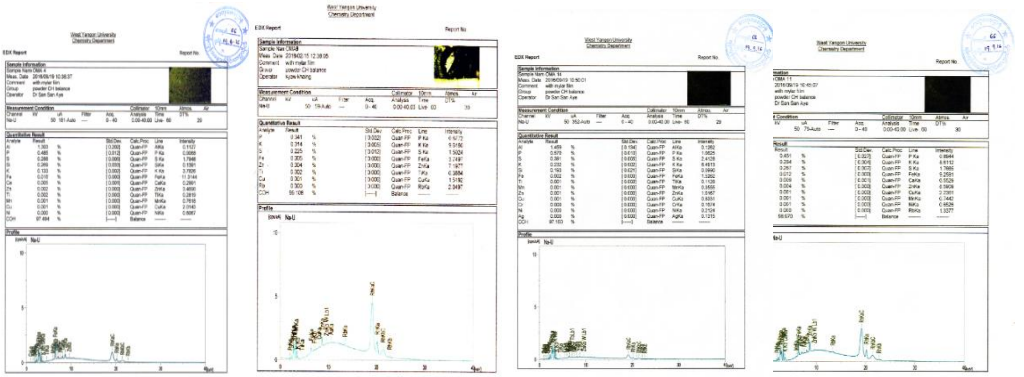
Figure 3: FT IR spectra of isolated fungi (a) OMA-4 (b) OMA-9 (c) OMA-11 (d) OMA-14

Table 6: FT IR Spectral Data of Metal-Tolerant Fungi

Observed Wave no. (cm^{-1})	Literature Wave no. (cm^{-1})	Band Assignment
3385,3417	3300-3600	υ_{O-H}, O-H and N-H stretching
2854, 2931	2800-2900	υ_{C-H}, (symmetric & asymmetric) of -CH_2, -CH_3 group
1558,1649	1600-1400	$\upsilon_{C=C,}$ stretching
1377,1416	1400-1300	$\delta_{C-H,}$ (symmetric & asymmetric) bending
1078,1149	1100-1020	$\upsilon_{C-O,}$ stretching

Characterization of Metal-Tolerant Fungi by EDXRF Analysis

The relative abundance of the elements comprising in samples were determined by EDXRF analysis. In EDXRF spectra, the major abundance of C-OH element were observed in metal-tolerant fungi. Potassium, sulfur and phosphorus were found in minor constituent elements and other elements such as zinc, copper and iron were observed in relatively small amount.



(a) (b) (c) (d)

Figure 4: EDXRF spectra of isolated fungi (a) OMA-4 (b) OMA-9 (c) OMA-11 (d) OMA-14

Table 7: Relative Abundance of the Elements in Metal Tolerance Fungi by EDXRF Analysis

Element	Result (%)			
	OMA-4	OMA-9	OMA-11	OMA-14
C-OH	97.494	99.108	98.970	97.150
Al	1.303	-	-	1.459
P	0.485	0.341	0.451	0.570
K	0.133	0.314	0.284	0.232
S	0.288	0.225	0.267	0.391
Si	0.269	-	-	0.193
Fe	0.015	0.005	0.012	0.002
Ca	0.005	-	0.009	-
Zn	0.002	0.004	0.004	0.001
Ti	0.002	0.002	-	0.001
Cu	0.001	0.001	0.001	0.001
Mn	0.001	-	0.001	0.001

Studies on Biosorption of Metal-Tolerant Fungi

The effect of pH on biosorption of Cu^{2+}, Ni^{2+}, and Cr^{6+} metal ions, which showed an increase in percent removal with an increase in pH of 3 to 5. Beyond pH 5, percent removal decreased with increase in pH (Table 8, Figure 5). This is due to the strong relations of biosorption to the number of surface negative charge, which depends on the dissociation of functional group. When pH is increased, there is reduction in the electrostatic attraction between the metal ions and the sorbent surface causes the decrease in percent removal.

The adsorbent dose of biomass varied with percent removal of metal ions were shown in Table 9 and Figure 6. The maximum adsorption of Cu^{2+} and Cr^{6+} were observed 43.55 % and 39.59 % at 0.5 g of optimum dose and for Ni^{2+} was the maximum adsorption 40.62 % at 0.4 g biomass dose. This may be due to the adsorption of metal ions to the surface of the cell increase gradually till they reached equilibrium value.

The removal percent on effect of metal ions concentration were shown in Table 10 and Figure 7. It was found that percent removal increased gradually and it was decrease with increase in metal ions concentration. The trend observed in graph might be explained by the ability of active sites to fully adsorb the metal ions at lower concentrations, whereas higher concentrations caused saturation of adsorption sites.

Table 11 and Figure 8 shows adsorption rates were high for the initial contact time and decreases gradually to approach equilibrium. The equilibrium time was observed at 8 h for Cr^{6+} and 6 h for both Cu^{2+} and Ni^{2+} adsorptions. It may be explained by initial rapid uptake due to surface adsorption and subsequent slow uptake due to the specific sites are saturated with more contact time.

Table 8: Effect of pH on Cu^{2+}, Ni^{2+} and Cr^{6+} Removal by OMA-9, OMA-14 and OMA-11

pH	% Removal		
	Cu^{2+} (OMA-9)	Ni^{2+} (OMA-14)	Cr^{6+} (OMA-11)
3	28.57	20.25	29.09
4	37.50	28.92	48.97
5	38.23	29.62	50.00
6	31.50	30.93	25.02
7	30.00	20.28	15.38
8	25.88	11.76	13.30

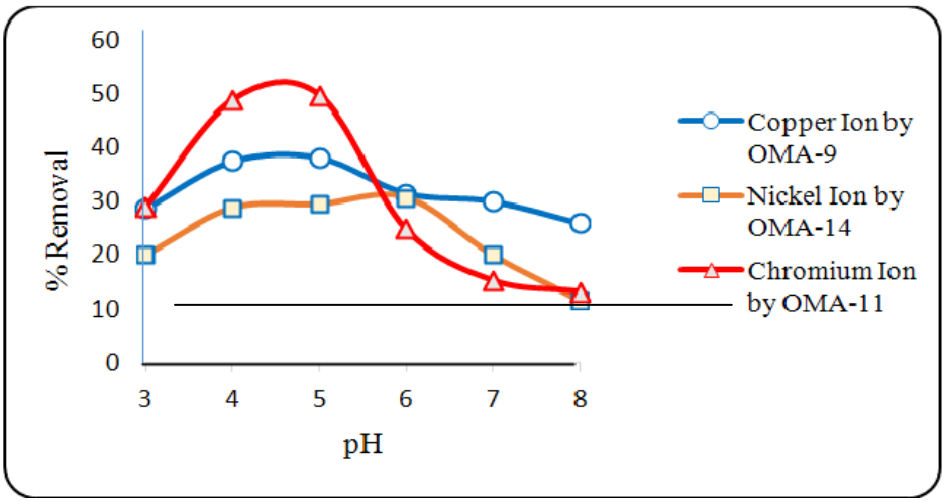


Figure 5: Effect of pH on removal of Cu^{2+}, Ni^{2+} and Cr^{6+} ions by OMA-9, OMA-14 and OMA-11 respectively

Table 9: Effect of Biomass Dosage on Cu^{2+}, Ni^{2+} and Cr^{6+} Removal by OMA-9, OMA-14 and OMA-11

Dosage (g)	% Removal		
	Cu^{2+} (OMA-9)	Ni^{2+} (OMA-14)	Cr^{6+} (OMA-11)
0.1	30.64	30.10	31.78
0.2	35.48	31.87	32.55
0.3	38.70	38.99	34.10
0.4	43.54	40.62	36.43
0.5	43.55	40.11	39.59

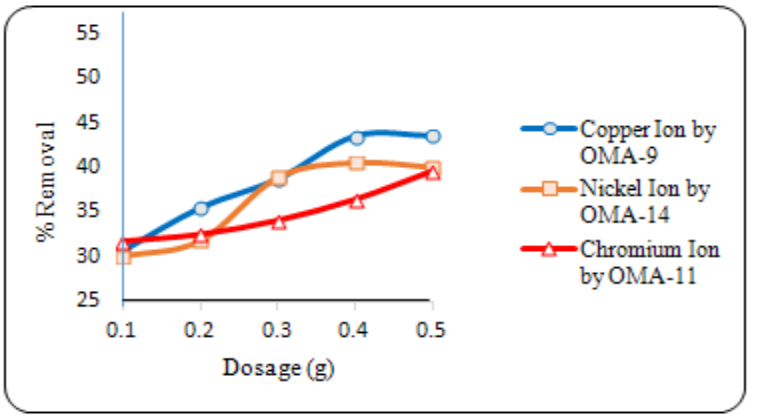


Figure 6: Effect of dosage on removal of Cu^{2+}, Ni^{2+} and Cr^{6+} ions by OMA-9, OMA-14 and OMA-11 respectively

Table 10: Effect of Metal Concentration on Cu^{2+}, Ni^{2+} and Cr^{6+} Removal by OMA-9, OMA-14 and OMA-11

Concentration (mM)	% Removal		
	Cu^{2+} (OMA-9)	Ni^{2+} (OMA-14)	Cr^{6+} (OMA-11)
10	43.75	40.74	38.84
20	55.78	42.04	39.84
30	53.54	42.10	41.17
40	50.09	40.21	35.50
50	50.10	37.69	34.04

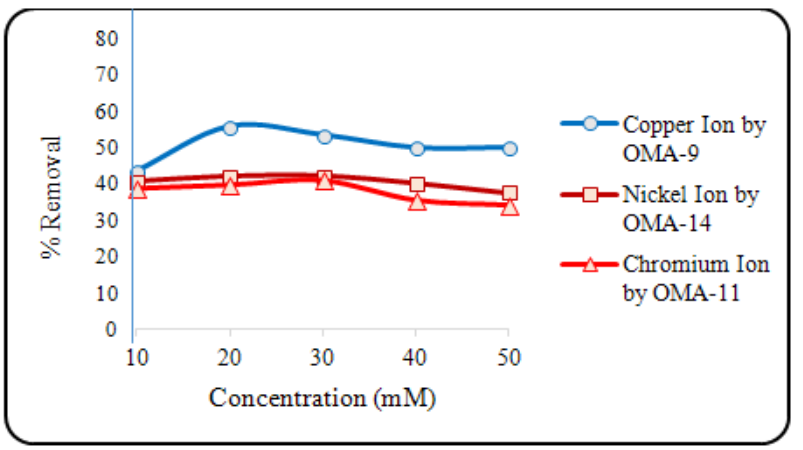


Figure 7: Effect of concentration on removal of Cu^{2+}, Ni^{2+} and Cr^{6+} ions by OMA-9, OMA-14 and OMA-11 respectively

Table 11: Effect of Contact Time on Cu^{2+}, Ni^{2+} and Cr^{6+} Removal by OMA-9, OMA-11 and OMA-14

Contact Time (h)	% Removal		
	Cu^{2+} (OMA-9)	Ni^{2+} (OMA-14)	Cr^{6+} (OMA-11)
2	48.70	41.76	41.30
4	49.47	44.70	42.02
6	56.44	45.29	42.75
8	56.35	44.11	43.47
10	55.44	40.58	40.54

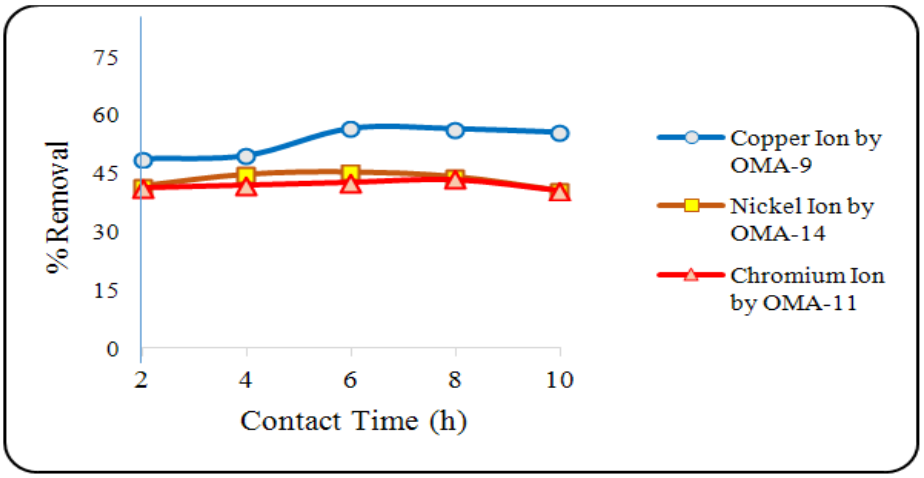


Figure 8: Effect of contact time on removal of Cu^{2+}, Ni^{2+} and Cr^{6+} ions by OMA-9, OMA-14 and OMA-11 respectively

Conclusion

In this experiment, fourteen different fungi were isolated from different soil samples at Pathein Township. Among them, OMA-9, OMA-11 and OMA-14 showed metal-tolerant activities of Cu^{2+}, Cr^{6+} and Ni^{2+} metal ions respectively. The biomass production of metal-tolerant fungi were also studied by varying time course on cultivation, pH, carbon source and nitrogen source consumption.

The metal removal by biosorption studies showed that their removal potential were different in different metal ions and operating conditions. The optimum conditions for Cu^{2+} ion removed by OMA-9 were 0.5 g of biomass

dose, 2 h of contact time, 20 mM of metal ion concentration and pH 5 were found. The Ni^{2+} ion removed by OMA-14 was observed at optimum condition of adsorbent dose 0.4 g, contact time 6 h, metal ion concentration 30 mM and pH 6. The biosorption of Cr^{6+} ion using OMA-11 was found to be biomass dosage 0.5 g, contact time 6 h, metal ion concentration 30 mM and pH 5. Therefore, the present study showed that chemically pretreated fungal biomass of OMA-9, OMA-11 and OMA-14 exhibited the tolerance activities and potential for removal of Cu^{2+}, Ni^{2+} and Cr^{6+} ions from aqueous solutions.

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