BIOSORPTION OF COPPER AND NICKEL IONS BY NON-LIVING BIOMASS OF *ASPERGILLUS* SPECIES

Ohnmar Aye[1], Ye Myint Aung[2]

Abstract

Two different fungi were isolated from agricultural soil in Pathein Township. Both isolates were studied on the basis of morphological and microscopical characteristics. Pure cultures of fungal isolates were identified with the help of literature keys of Ando (2016). According to morphology and distinct characters, the two fungal strains were identified as *Aspergillus* sp. 1 and *Aspergillus* sp. 2. The two *Aspergillus* species were tested for their tolerance against heavy metals such as Cu^{2+} and Ni^{2+} ions at different concentration of 10 mM, 20 mM, 30 mM, 40 mM and 50 mM. It was observed that *Aspergillus* sp. 1 tolerance against Cu^{2+} ion and *Aspergillus* sp. 2 tolerance against Ni^{2+} ion. The ability of isolated fungal strains towards biosorption potential of metal ions from industrial wastewater sample were also studied. In this study, the adsorption efficiency of biomass was determined by using the functions of contact time and biomass dose. The biosorption capacity of *Aspergillus* sp. 1 reached 47.58 % removal of Cu^{2+} ion, while *Aspergillus* sp. 2 was expressed 39.04 % removal of Ni^{2+} ion. The finding revealed that fungi of two *Aspergillus* species showed higher metal-tolerant and biosorption capacity of copper and nickel ions from wastewater.

Keywords: metal-tolerant, biosorption, *Aspergillus* sp., contact time

Introduction

Heavy metal pollution is a serious environmental problem of global concern. Heavy metals are continuously released into the environment due to industrial and technological developments, and contamination of agricultural soil with heavy metals is a major problem at industrial and defense-related sites all over the world. The industrial effluents are generated from hundreds of small and large manufacturing and plating industries such as metallurgical, electroplating, metal finishing, tanneries, chemical manufacturing, mine drainage, and battery manufacturing, and contain considerable amounts of heavy metals at elevated concentrations. (Malik and Jaiswal, 2000).

Filamentous fungi and yeasts have been used in many wastewater treatments to bind metallic elements. Fungi are one of the industrial fermentation waste biomass which is really excellent metal sorbed. So, fungi including yeasts have received increased attention. Fungi gives good efficient and economical for sequestering heavy toxic metals from dilute aqueous solutions by biosorption because: it offers the advantage of having a high percentage of cell wall materials, it shows excellent metal binding properties, it is available in large quantities from the antibiotic and food industries, it provides an eco-friendly environment (Gadd, 1993).

Traditional methods used for the removal of heavy metals from the environment are in general expensive and potentially risk due to the possibility of the generation of hazardous by-products. For example, the use of conventional technologies, such as ion exchange, chemical precipitation, reverse osmosis, and evaporative recovery, for this purpose is often inefficient and very expensive. In recent years, the biosorption process has been studied extensively using microbial biomass as biosorbents for heavy metal removal (Gavrilesca, 2004).

[1] Dr, Lecturer, Department of Chemistry, Pathein University
[2] Dr, Professor and Head, Department of Chemistry, Pathein University

The main purpose of the present study was characterization of metal-tolerant fungi isolated from soil samples and selection of more resistant strains. The reason for the selection of the fungi is that the organisms that usually adapt the conditions of metals and in future these fungi could be used as biosorption tool.

Materials and Methods

Isolation of Soil Fungi by Serial Dilution Method

In serial dilution method, 0.1 g of dried soil sample was diluted with 10 mL 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).

Media used for the isolation of soil fungi

(1) Low Carbon Agar (LCA) Medium (Ando, 2004)		**(2) Potato Glucose Agar (PGA) Medium (Ando, 2004)**	
Glucose	2.0 g	Potato	200 g
Sucrose	2.0 g	Glucose	20 g
K_2HPO_4	1.0 g	Agar	18 g
$MgSO_4.7H_2O$	0.5 g	Yeast Extract	1.0 g
KNO_3	1.0 g	DW	1.0 L
KCl	0.5 g	pH	6.5
Agar	18 g		
DW	1.0 L		
pH	6.5		

(After autoclaving chloramphenicol 0.03 g was added to both media)

Identification of Isolated Fungi

Fungal isolate were studied for its morphological features under light microscope at Botany Department, Pathein University. The cultures were identified on the basis of macroscopic (colonial morphology, colour, shape, diameter and colony appearance) and microscopic characteristics (septation in mycelium, shape and structure of conidia). The mycelia were observed at visual inspection. The microscopical characters including conidiophore, vesicle, conidia and hyphae were observed.

Determination of Metal-Tolerant Fungi by Spot Culture Method

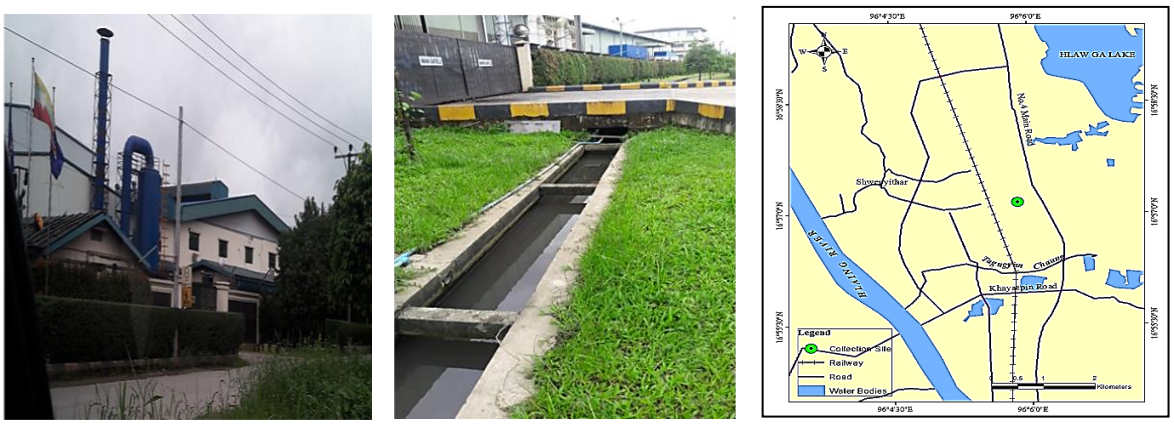
A disk of mycelium was inoculated aseptically on potato glucose agar (PGA) plates supplemented individually with 10, 20, 30, 40 and 50 mM of heavy metal. The inoculated plates were incubated at 25 °C at least for 15 days. Three replicates for each of concentrations and control (medium without metal) were used. The effect of the heavy metal on the growth of the isolates was estimated by minimum inhibitory concentrations (MICs). MIC is defined as the lowest concentrations of metal that inhibit visible growth of the isolate (Hassen and Saidi, 1998).

Study on Biosorption of Toxic Heavy Metals from Industrial Wastewater

The biosorption process was generally applied for the toxic heavy metals (Cu^{2+} and Ni^{2+} ions) removal from industrial wastewater.

Industrial wastewater sample was collected from Shwe Lin Ban Industrial Zone, Shwe Phyi Thar Township, Yangon (Figure 1). The wastewater samples were taken at a distance of about 1 m from the point source of drainage and at a depth of 0.2 m below the surface of water with sterilized prewashed polyethylene container. The initial concentrations of Cu^{2+} and Ni^{2+} ions in the wastewater were analyzed by Atomic Absorption Spectrophotometer (AAS) from Ministry of Education, Department of Research and Innovation (DRI), Yangon.

In this study, the fungal species *Aspergillus* sp. 1 and *Aspergillus* sp. 2 were used as biosorbent for removal of metal ions from wastewater. The heavy metal removal was determined by the effect of biomass dose and contact time.



(a) (b) (c)

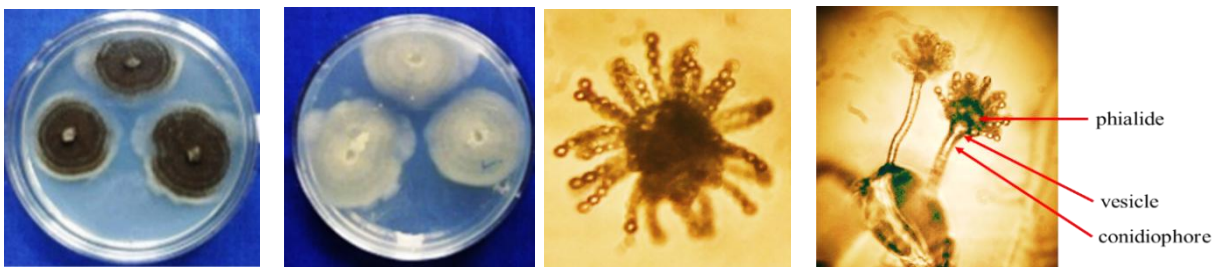
Figure 1 Wastewater sample collected sites

(a) Battery factory (b) Drainage of wastewater (c) Location map

Results and Discussion

Identification of Isolated Fungi

Morphological and microscopical descriptions of OMA-1



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

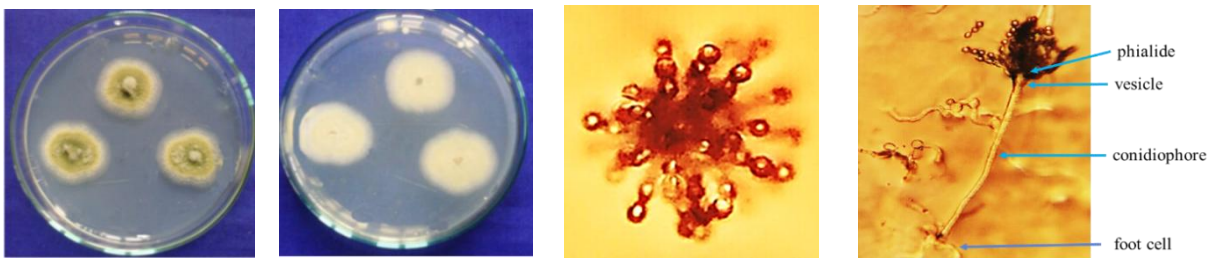
Figure 2 Micromorphological characters of OMA-1

(a) Front view (b) Reverse view (c) Conidia (d) Microscopical description

The colonies are fast growing and mature within 3 days. The colonies are flat and powdery in texture. The front colour of the colony is black center and white edge. The reverse colour is whitish grey or creamy (Figure 2).

The hyphae are septate and hyaline. The conidiophores stipes are smooth-walled, hyaline and erect. The conidiophores terminate in a spherical to pyriform vesicle. The vesicle, phialide, metulae and conidia are the parts to form the conidial head. The conidia are globose to subglobose, one-celled, smooth-walled, dark brown to black are produced in long chains.

Morphological and microscopical description of OMA-2



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

Figure 3 Micromorphological characters of OMA-2
(a) Front view (b) Reverse view (c) Conidia (d) Microscopical description

The colonies are flat often with granular, yellow at first but quickly becoming bright to dark yellow-green with age. The reverse colour is whitish or creamy (Figure 3).

The hyphae are septate. The large cell of the hyphae are known as foot-cells. From each foot cell arises a vertical branch, is called conidiophore. Each conidiophore elongates and end in a dome-shaped head called the vesicle. The sterigmata or phialides are the tubular outgrowths, which are produced directly from the vesicle. The conidia are produced in long basipetal chains, and is enormous numbers. The conidia are spherical and unicellular structure. The conidia are brownish in colour (Figure 3).

Identification key of OMA-1 and OMA-2

The OMA-1and OMA-2 fungus were identified as *Aspergillus* sp.1 and *Aspergillus* sp.2 according to keys of Ando, (2016) as follows. The results are summarized in Table 1.

1. Conidial Ontogeny (i) Conidial production is chain
   (ii) Type of conidial production is Phialo type
   (iii)Type of conidial ontogeny is Enteroblastic chain
2. Conidiophores - Typical conidiophores with septa, simple and unbranched.
3. Conidiophores - Elongate along with conidial production
4. Arrangement of Conidiogenous cells - Independent (Parallel)
5. Development of Conidiogenous cells - Stable
6. Conidial production loci of Conidiogenous cells - Mono
7. Conidia (i) Shape - Simple, (ii) Spore - Amerospore
8. Hyphae - with septa regularly
9. Identified these fungus as *Aspergillus* sp.

Scientific Classification

Kingdom	:	Fungi
Phylum	:	Ascomycota
Class	:	Eurotiomycetes
Order	:	Eurotiales
Family	:	Trichocomaceae
Genus	:	*Aspergillus* sp.

Table 1 Morphological and Physical Characteristics of the Isolated Fungal Species

Characteristics	***Aspergillus* sp.1**	***Aspergillus* sp.2**
Colony diameter	53.5 mm	55.04 mm
Conidial colour	Black	Brown
Conidial shape	Globose	Globose
Conidiophore colour	Brown	Brown
Mycellial colour	Black center with white edge	Yellowish green
Colonial reverse	Whitish grey	Cream
No. of sterigmata	Present in two series	Present in one series

Screening of Metal-Tolerant Fungi by Spot Culture Method

In this study, heavy metal- tolerant fungi were screened and the minimum inhibitory concentration of Cu^{2+} and Ni^{2+} were determined (Figures 4 and 5). The growth pattern appears to suggest tolerance development or adaptation of the fungi to the presence of heavy metals. In the presence of heavy metal relative to the control, the growth rate of the fungi decreased with increased in metal ions concentration. At lower metal ions concentrations, the tested fungal isolates were very resistant and exhibited strong growth. Higher metal ion concentrations caused a reduction in growth and increased the length of the lag phase compared to the control. A reduction in the growth rate is a typical response of fungi to toxicants, whereas the lengthening of the lag phase is not always present.

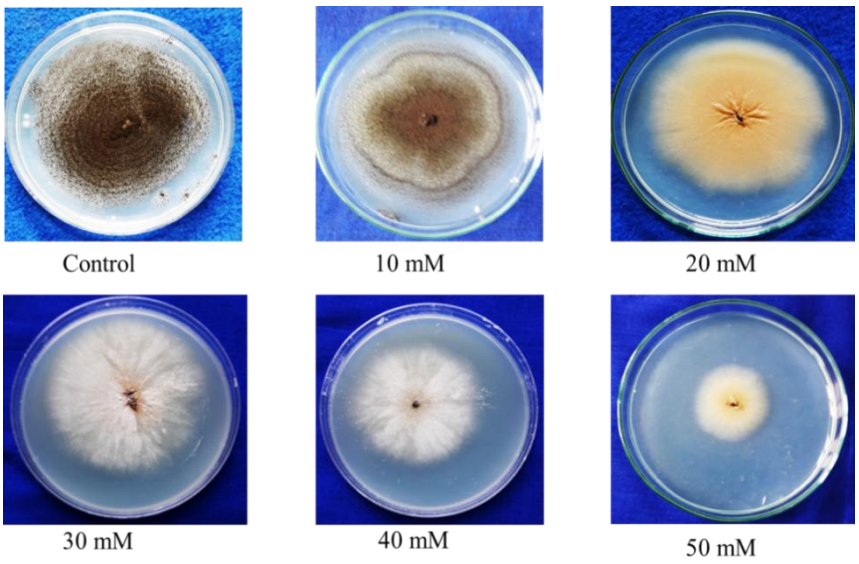


Figure 4 Growth of *Aspergillus* sp.1 after exposure to concentration of Cu^{2+} ion for 15 days

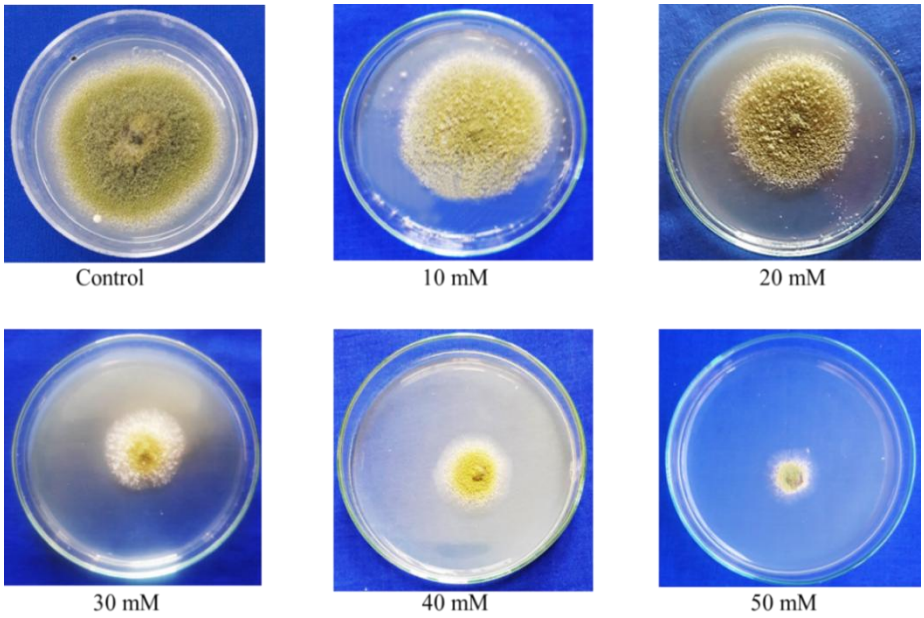


Figure 5 Growth of *Aspergillus* sp.2 after exposure to concentration of Ni^{2+} ion for 15 days

Study on Biosorption of Toxic Heavy Metals from Industrial Wastewater

Table 2 and Figure 6 showed the effect of biomass dose on percent removal of metal ions. It was obvious that removal percent increased with increased in dosage and significantly decreased at dosage 0.5 g. This may be due to the increase in surface area and number of available active site for adsorption of metal ions with saturation of cell surface.

The removal percent on effect of contact time are shown in Table 3 and Figure 7. It was found that percent removal increased gradually to the maximum adsorption and then decreased with increased in contact time. It may be explained by initial rapid uptake due to surface adsorption and subsequent slow uptake due to the specific sites are saturated with metal ions.

Table 2 Effect of Biomass Dosage on Removal of Cu^{2+} and Ni^{2+} Ions by Different Fungal Biosorbents

Contact time = 4 h
pH = 6.0

Dosage (g)	Removal (%)	
	Cu^{2+} (by *Aspergillus* sp. 1)	Ni^{2+} (by *Aspergillus* sp. 2)
0.1	42.74	32.49
0.2	43.27	34.50
0.3	46.77	35.01
0.4	47.58	39.04
0.5	43.80	36.02

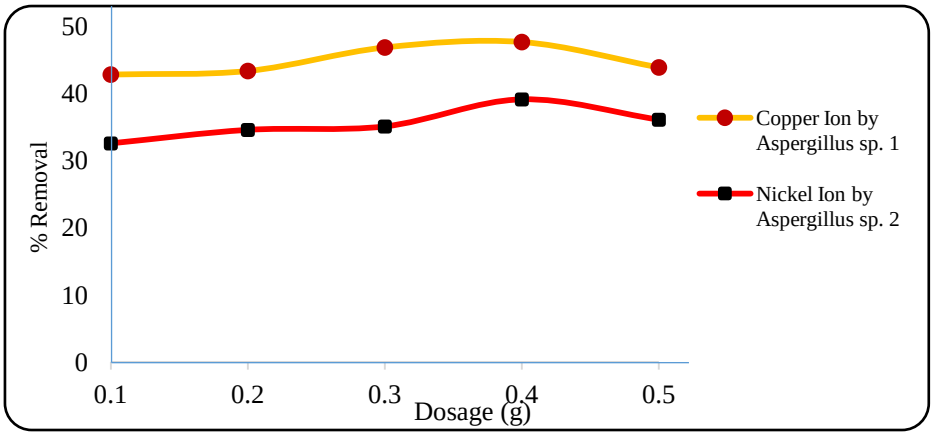


Figure 6 Effect of biomass dosage on removal of Cu^{2+} and Ni^{2+} ions by *Aspergillus* sp. 1 and *Aspergillus* sp. 2

Table 3 Effect of Contact Time on Cu^{2+} and Ni^{2+} Ions Removal by Different Fungal Biosorbents

Biomass dose = 0.4 g

pH = 6.0

Contact Time (h)	Removal (%)	
	Cu^{2+} (by *Aspergillus* sp. 1)	Ni^{2+} (by *Aspergillus* sp. 2)
2	48.11	28.96
4	48.38	31.23
6	49.46	32.24
8	49.19	31.48
10	48.92	30.73

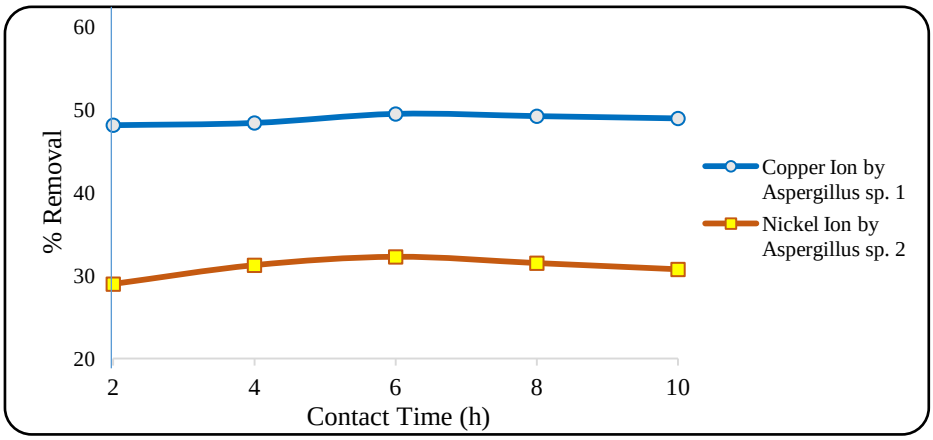


Figure 7 Effect of contact time on removal of Cu^{2+} and Ni^{2+} ions by *Aspergillus* sp. 1 and *Aspergillus* sp. 2

Adsorption Isotherm Assessment

Adsorption isotherm shows the distribution of solute between the liquid and solid phases and can be described by the standard Langmuir isotherms (Figures 8, 9 Tables 4, 5) and Freundlich models (Figures 10, 11 and Tables 6, 7).

Langmuir equation which is valid for monolayer sorption on to a surface with a finite number of identical sites and the linearized form of this model equation is given as

$$\frac{C_e}{q_e} = \frac{C_e}{q_{max}} + \frac{1}{(q_{max}\ b)}$$

Where C_e is the equilibrium concentration , q_{max} is the maximum amount of the metal ion per unit weight of the adsorbent to form a complete monolayer and b is a constant related to the affinity of the binding sites. q_{max} and b can be determined from the linear plot of C_e /q versus C_e.

The empirical Freundlich model also considers mono molecular layer coverage of solute by the adsorbent.

$$\log q = \log K + \frac{1}{n} \log C_f$$

where, K and n are the Freundlich constants characteristics of the system. The q_{max} value of these isotherm model reflects the metal affinity to the sites of biomass. That is the number of metal ions which form a complete monolayer on the surface of the biomass. The linearized Langmuir and Freundlich adsorption isotherm parameters are shown in Table 8 with the value of linear regression coefficients. The coefficients of determination (R^2) are 0.9973 and 0.9920 for Cu^{2+} and Ni^{2+} ions respectively. The values for linear regression (R^2) indicated that the adsorption nature is well fitted with both models.

Table 4 Langmuir Isotherm Data for the Biosorption of Cu^{2+} Ion by *Aspergillus* sp.1

C_e (mmol L^{-1})	q_e (mmol g^{-1})	C_e/q_e (g L^{-1})
8.18	9.05	0.91
8.24	4.39	1.87
8.27	2.87	2.88
8.33	2.08	3.99
8.38	1.61	5.18

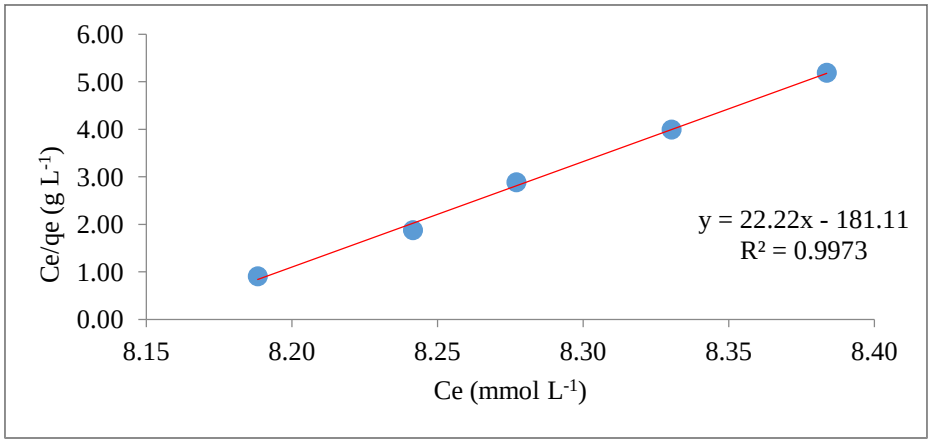


Figure 8 Langmuir adsorption isotherm of Cu^{2+} ion by *Aspergillus* sp.1

Table 5 Langmuir Isotherm Data for the Biosorption of Ni^{2+} Ion by *Aspergillus* sp.2

C_e (mmol L^{-1})	q_e (mmol g^{-1})	C_e/q_e (g L^{-1})
7.11	14.45	0.49
7.12	7.20	0.98
7.13	4.78	1.49
7.14	3.57	1.99
7.15	2.85	2.50

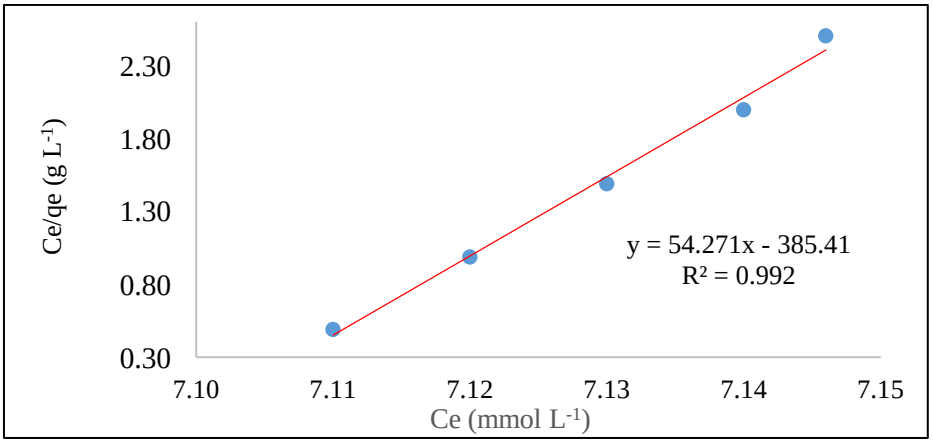


Figure 9 Langmuir adsorption isotherm of Ni^{2+} ion by *Aspergillus* sp.2

Table 6 Freundlich Isotherm Data for the Biosorption of Cu^{2+} Ion by *Aspergillus* sp.1

C_e (mmol L^{-1})	q_e (mmol g^{-1})	log C_e (mmol L^{-1})	log q_e (mmol L^{-1})
8.188	9.0586	0.913	- 0.039
8.241	4.596	0.916	- 0.038
8.277	2.871	0.917	- 0.037
8.330	2.087	0.920	- 0.035
8.383	1.616	0.923	- 0.034

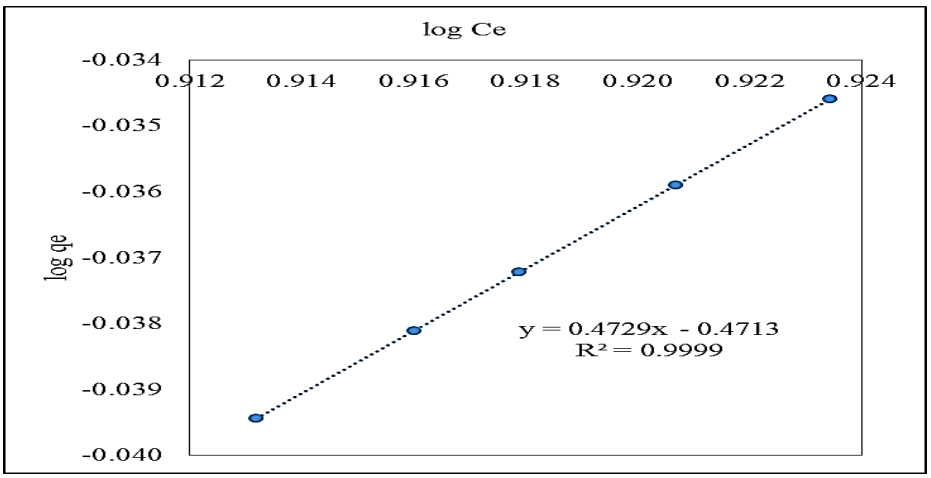


Figure 10 Freundlich adsorption isotherm of Cu^{2+} ion by *Aspergillus* sp.1

Table 7 Freundlich adsorption isotherm of Ni^{2+} ion by *Aspergillus* sp.2

C_e (mmol L^{-1})	q_e (mmol g^{-1})	log C_e (mmol L^{-1})	log q_e (mmol L^{-1})
7.1100	14.4500	0.8519	-0.0696
7.1200	7.2000	0.8525	-0.0693
7.1300	4.7833	0.8531	-0.0690
7.1400	3.5750	0.8537	-0.0687
7.1460	2.8540	0.8541	-0.0685

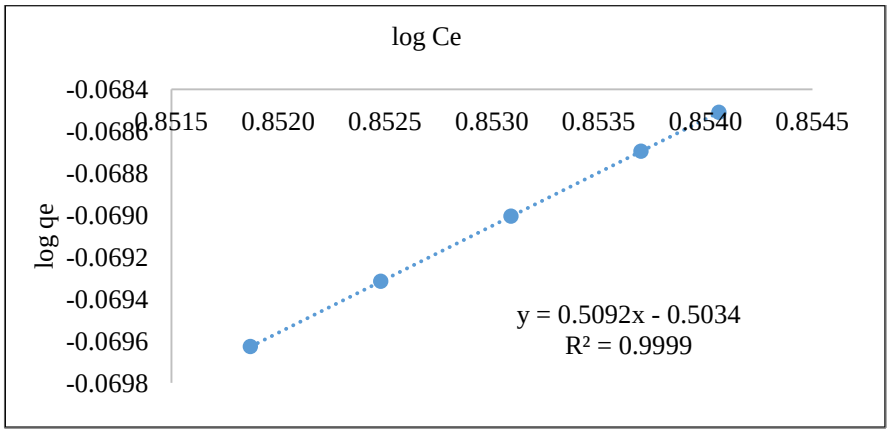


Figure 11 Freundlich adsorption isotherm of Ni^{2+} ion by *Aspergillus* sp.2

Table 8 Isotherm Parameters for the Biosorption of Metal Ions

Metal Ions	Biomass	Langmuir Model			Freundlich Model		
		q_{max} (mg g^{-1})	R^2	b (L mg^{-1})	K_f	R^2	N
Cu^{2+}	*Aspergillus* sp.1	2.8599	0.9973	0.0019	0.3378	0.9999	2.1146
Ni^{2+}	*Aspergillus* sp.2	1.0814	0.9920	0.0024	0.2876	0.9999	1.8002

Conclusion

Soil fungi were isolated from agriculture soil samples by using serial dilution method whereas LCA and PGA media were used. Morphological and microscopic characters of the isolated fungi were identified by using keys of Ando (2016). According to morphology and distinct characters, OMA-1 fungus was identified as *Aspergillus* sp. 1, OMA-2 fungus was identified as *Aspergillus* sp. 2.

Fungal isolates were tested for their tolerance against different concentrations of metal ions such as Cu^{2+} and Ni^{2+}. *Aspergillus* sp.1 was most tolerant to metal concentration of copper, and different isolates of *Aspergillus* sp.2 was tolerant to nickel ion at MIC 50 mM. This means that the levels of resistance are different among different isolates.

In the biosorption study of wastewater treatment, the industrial wastewater was collected and initial metal ion concentrations of copper and nickel in collected sample were analyzed. The

biosorption of heavy metals by using different biomass showed different results. According to results, ability of *Aspergillus* sp. 1 was found to adsorb the copper ion with excellent properties and maximum removal of 49.46 % at optimum dose 0.4 g and contact time 6 h. The adsorption capacity of *Aspergillus* sp. 2 was expressed as 32.24 % removal of nickel ion at 0.4 g of adsorbent dose and 6 h contact time.

The adsorption quantity was evaluated by Langmuir and Freundlich models. The plot of C_e/q_e Vs C_e in various biomass dose was found to be linear indicating the applicability of classical Langmuir adsorption isotherm. The coefficients of determination (R^2) are 0.997 and 0.992 for Cu^{2+} and Ni^{2+} ions respectively. The values for linear regression (R^2) indicated that the adsorption nature is well fitted with Langmuir model. The higher metal adsorption of 2.8599 mg g^{-1} was observed for Cu^{2+} ion whereas the lower uptake was 1.0814 mg g^{-1} for Ni^{2+} ion. For the Freundlich model, log q_e vs log C_e was plotted and K_f and n values are Freundlich constants related to the adsorption capacity and intensity of the sorbents, respectively. The sorption capacity parameter K_f was observed that 0.3378 for Cu^{2+} ion and 0.2876 for Ni^{2+} ion respectively. The results of this study revealed that the fungal cells of *Aspergillus* sp. 1 and *Aspergillus* sp. 2 have greater potential application for the removal of copper and nickel ions from industrial wastewater sample.

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