ISOLATION, IDENTIFICATION AND CHROMIUM ADSORPTION BEHAVIOUR OF A CHROMIUM-RESISTANT *PAECILOMYCES* sp.

Ohnmar Aye[1], Khin Myo Myint[2]

Abstract

The present study was carried out to investigate metal resistant and adsorption strain. A chromium-resistant fungus M-1 was isolated from soil sample by conventional plating method. The isolated fungus was studied on the basis of morphological, microscopical characteristics and identified with the help of literature keys of Ando (2016). According to morphology and distinct characters, the fungal strain was identified as *Paecilomyces* sp. The fungal growth in metal resistant against Cr^{6+} ion at different concentration of 10 mM, 20 mM, 30 mM, 40 mM and 50 mM was studied. It was observed that *Paecilomyces* sp. was found to be resistant against 50 mM of Cr^{6+} ion. The biomass of chromium-resistant fungus was used for biosorption experiment by varying contact time and biomass dose. The biosorption of Cr^{6+} ion from industrial wastewater by chromium- resistant biomass showed 45.48 % removal and 1.9058 mg g^{-1} of Cr^{6+} ion at optimum contact time 8 h and biomass dose 0.4 g respectively. From the results, that *Paecilomyces* sp. showed chromium-resistant behaviour and biosorption potential for removal of Cr^{6+} ion from industrial wastewater.

Keywords: chromium- resistant, biosorption, contact time, biomass

Introduction

Microbes often have other genetically determined defences against harmful metals when they cannot detoxify them. Fungi can be screened as potential bioremediation agents due to their greater growth capacity and reach by virtue of mycelial branching, a greater potential to produce a number of enzymes, and they are good accumulators of various metals. Exploration of various habitats may lead to fungal strains with diverse potentials.

Industrial and metallurgical processes release a wide range of toxic metal pollutants as their waste products. Chromium is one of the metals of most immediate concern according to the World Health Organization (1984). Chromium may be present in effluent in various chemical forms. Hexavalent chromium compounds tend to be more mobile and toxic than trivalent chromium compounds (Calder, 1988) while Cr(VI) may be detrimental to human beings and animals and have pronounced adverse effects on plants and aquatic life. Most of the current methods of chromium removal are expensive and inconsistent, and may generate toxic sludge that requires careful disposal (Wild, 1987). Bioremediation using various microorganisms can be a more promising alternative than chemical treatment of such toxicants. Microbes (both prokaryotes and eukaryotes) have the ability to bind metal ions in the external environment at the cell surface, or to transport them into the cell. Some may form metabolic products such as acids or ligands that dissolve base metals dissolved in mineral or anions such as sulfide or carbonate that precipitate dissolved metal ion (Ehrlich, 1997).

The aim of the study was to examine the potential of metal tolerant fungal strain associated with metal tolerant and accumulate hexavalent chromium, one of the most hazardous heavy metals, its effect on fungal biomass production and thus, to examine their application as biosorption process for wastewater treatment.

[1] Dr, Associate Professor, Department of Chemistry, Dawei University
[2] Dr, Professor, Department of Botany, Loikaw University

Materials and Methods

Media Used for Isolation

(1) Beef-extract Peptone Medium (Ando, 2004)		(2) Potato Dextrose Agar (PDA) Medium (Ando, 2004)	
Beef-extract	3.0 g	Potato	200 g
Peptone	10.0 g	Dextrose	20 g
NaCl	10.0 g	Agar	18 g
DW	1.0 L	DW	1.0 L
pH	6.5	pH	6.5

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

Isolation of Chromium-Resistant Fungi

Chromium-resistant fungus was isolated by using conventional plate method. The stock solution of Cr^{6+} ion containing concentration of 1000 mgL^{-1} was prepared by dissolving 2.829 g of potassium dichromate, $K_2Cr_2O_7$ in 1000 mL of deionized water and this solution was further diluted with water to desired concentration of test solution. Soil suspension was added to beef-extract peptone liquid medium containing 2mM of Cr^{6+} ion solution. The cultures were incubated on a rotatory shaker at room temperature, 180 rpm for 3-5 days. The spore suspension was spotted on metal containing agar plates (PDA). The plates were incubated at 25°C for 7 days and the growth of the chromium-resistant fungus M-1 was observed.

Identification of Isolated Fungi

Fungal isolate was studied for its morphological features under light microscope. The culture was identified on the basis of morphological (colonial morphology, colour, shape, diameter and colony appearance) and microscopical characteristics (septation in mycelium, shape and structure of conidia).

Metal-Tolerant Test for Fungi

The metal tolerance of fungi was determined by liquid (PDB) and solid (PDA) media. In liquid medium, 1 mL inoculum (spore suspension) was inoculated in 500 mL of Potato Dextrose Broth medium containing different concentrations of Cr^{6+} i.e., 25, 50 and 100 mM, as potassium dichromate. Each set was prepared in triplicate. One set of medium without Cr^{6+} was also inoculated, which was kept as control. The inoculated flasks were incubated at room temperature for 15 days and effect of chromium on biomass (and in that way tolerance to Cr^{6+}) was recorded in terms of dry weight of the inoculated fungi.

A series of solid PDA media which contained different concentrations of 10, 20, 30, 40 and 50 mM of Cr^{6+} ion. The spore suspension was spotted on metal containing agar plates. The strain was orderly inoculated and cultivated from low concentration to high concentration level. The inoculated plates were incubated at 25 °C at least for 15 days to observe the growth of fungus with chromium-resistant. Three replicates for each concentration were recorded. The PDA medium without metal was also inoculated as a control plate. The concentration which fungal isolates fail to grow was estimated as minimum inhibitory concentration. 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 Metal Cr^{6+} Ion from Industrial Wastewater

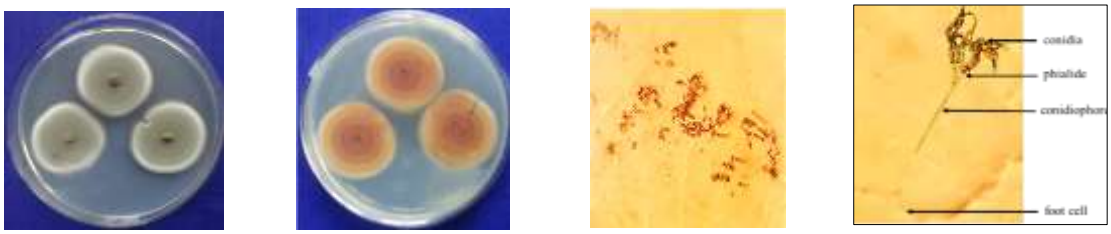
The biosorption process was generally applied for the removal of toxic heavy metal Cr^{6+} ion from industrial wastewater.

Industrial wastewater sample was collected from Shwe Lin Ban Industrial Zone, Shwe Phyi Thar Township,Yangon in November, 2018. The wastewater sample was 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 concentration of Cr^{6+} ion in the wastewater was analyzed by Atomic Absorption Spectrophotometer (AAS) from Ministry of Education, Department of Research and Innovation (DRI), Yangon.

In this study, the fungal isolate *Paecilomyces* sp. was used as biosorbent for removal of Cr^{6+} metal ion from industrial wastewater. The heavy metal removal was determined by the effect of biomass dose (0.1 g, 0.2 g, 0.3 g, 0.4 g and 0.5 g) and contact time (2 h, 3 h, 4 h, 6 h, 8 h, and 10 h).

Results and Discussion

Identification of Isolated Fungi



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

Figure 1 Morphological characters of isolated fungi (a) Front view (b) Reverse view (c) Conidia (d) Microscopical description

Morphological and Microscopical Description of M-1

The colonies of isolated fungus M-1 grow rapidly and mature within 3 days. The colonies are flat and powdery or velvety in texture. The colour is initially white and becomes pale green or olive brown colour. The reverse colour is yellowish brown (Figure 1 a, b).

The microscopic character showed mycelia had septate hyaline hyphae and branches. Slender conidiophores are grown out from mycelia. The conidia are long, dry chains of single-celled, smooth or rough hyaline to darkly coloured, ovoid conidia are produced in the basipetal succession from the phialides (Figures 1 c, d). The phialides of this fungus taper towards their apices and are organized slightly apart from each other. The phialides of *Penicillium* have thicker apices and are organized in tight clusters. Colonies of *Penicillium* are commonly blue-green in colour while those of *Paecilomyces* are not (Samson, 1974).

Identification key of isolated fungi

The isolated fungus was identified as *Paecilomyces* sp. according to keys of Ando, (2016) as follow.

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 branches
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 this fungus as *Paecilomyces* sp.

Scientific Classification

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

Table 1 Morphological and Physical Characteristic of the Isolated Fungi

Characteristics	*Paecilomyces* sp.
Colony diameter	42.3 mm
Conidial colour	Brown
Conidial shape	Elliptic or ovoid
Conidiophore colour	Hyaline
Mycellial colour	Pale green
Colonial reverse	Brownish yellow
No. of sterigmata	Present in one series

Screening of Metal-Tolerant Fungi

Figure 2 Growth of *Paecilomyces* sp. after exposure to concentration of Cr^{6+} ion in PDB medium

In this study, chromium-tolerant fungus was screened by PDB and PDA medium. Fungus *Paecilomyces* sp. showed luxuriant growth in PDB medium containing Cr^{6+}ion. Effect of Cr^{6+}ion on growth of fungi was estimated in terms of dried weight of biomass (Figure 3). The biomass was dried at 60º C to obtain constant weight. The results suggested that biomass production was higher in control and lower in Cr^{6+}metal ion treated media.

Table 2 Dry Cell Weight of Fungal Biomass *Paecilomyces* sp.

No.	Cr^{6+} Concentration (mM)	Dry weight (g/100 mL)
1	Control	1.234
2	25	0.523
3	50	0.427
4	100	0.211

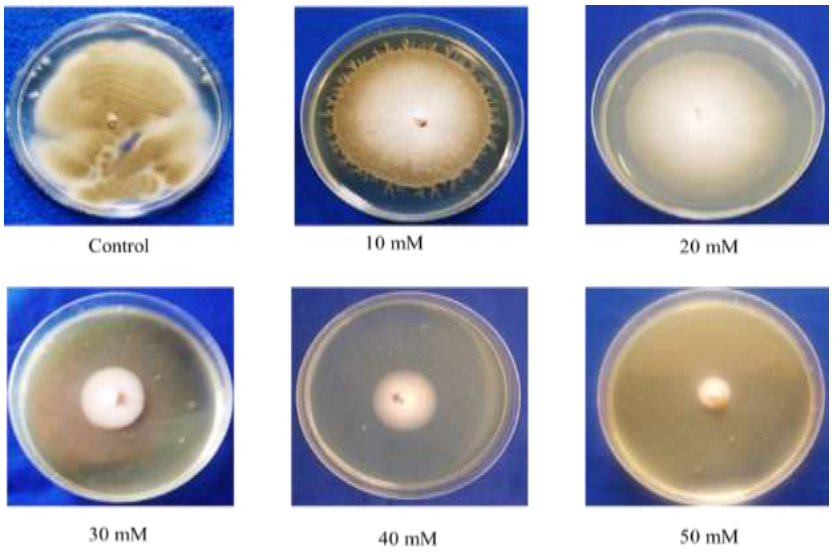


Figure 3 Growth of *Paecilomycess* sp. after exposure to different concentration of Cr^{6+} ion in PDA medium

In solid PDA medium, *Paecilomyces* sp. appeared white density mycelia and maximum growth of isolates at 10 mM. When the metal ion concentration gradually increased, the fungal cultural growth was inhibited. Then the metal concentration reached 50 mM, the fungi can failed to grow after culturing for 15 days, and 50 mM was thought as the MIC of Cr^{6+} ion. It was found that the low concentration of the metal, less toxicity to fungal cell whereas higher concentration of metal, high toxicity to the cell and more sinhibited the cell growth.

Study on Biosorption of Toxic Heavy Metal Cr^{6+} Ion from Industrial Wastewater

Table 3 Effect of Biomass Dosage on Removal of Cr^{6+} Ion by *Paecilomyces* sp.

Contact time = 8 h
pH = 6.0
Temperature = 30˚C

Dosage (g)	Initial Concentration (mg/L)	Final Concentration (mg/L)	Percent removal
0.1	0.62	0.394	36.45
0.2	0.62	0.380	38.71
0.3	0.62	0.356	42.58
0.4	0.62	0.338	45.48
0.5	0.62	0.344	44.51

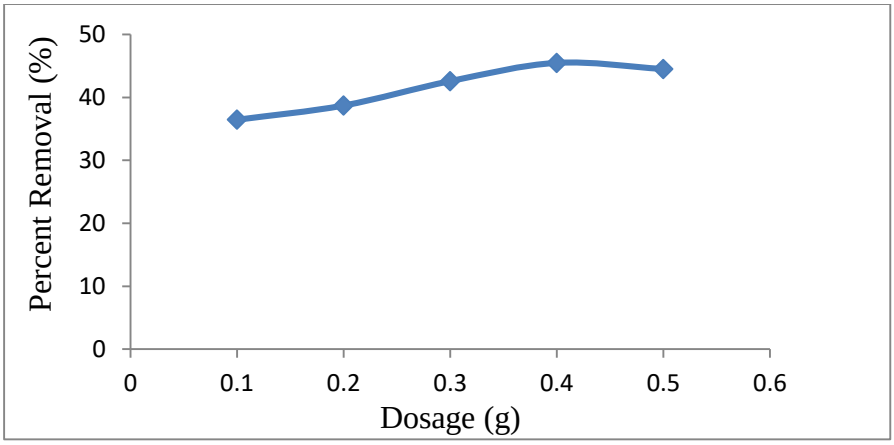


Figure 4 Effect of biomass dosage on removal of Cr^{6+} ion by *Paecilomyces* sp.

Table 4 Effect of Contact Time on Removal of Cr^{6+} Ion by *Paecilomyces* sp.

Biomass dose = 0.4 g
pH = 6.0
Temperature = 30˚C

Contact Time (h)	Initial concentration (mg/L)	Final concentration (mg/L)	Percent removal
2	0.62	0.380	38.70
4	0.62	0.368	40.64
6	0.62	0.357	42.41
8	0.62	0.338	45.48
10	0.62	0.359	42.09

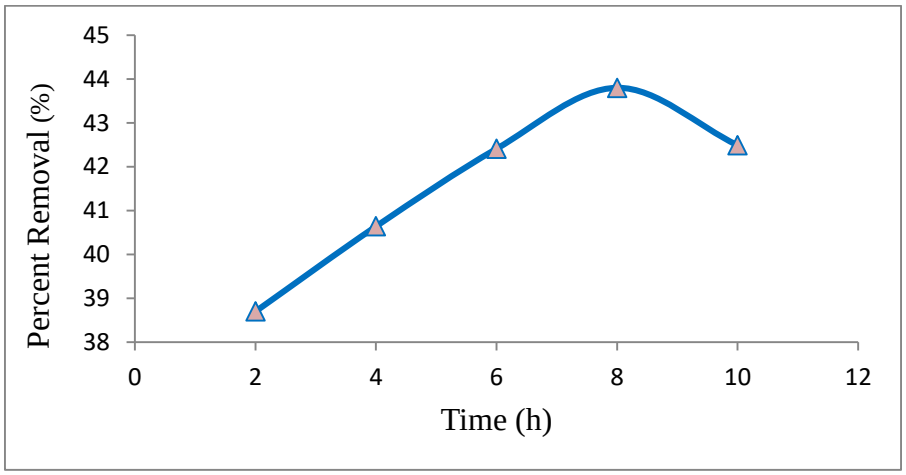


Figure 5 Effect of contact time on removal of Cr^{6+} ions by *Paecilomyces* sp.

Table 3 and Figure 4 showed the effect of biomass dose on percent removal of metal ions. It was observed 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 4 and Figure 5. It was found that percent removal increased gradually to the maximum adsorption and then to attain

equilibrium 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 is saturated with metal ions.

Adsorption Isotherm Assessment

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.

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

Where, K and n are the Freundlich constants characteristics of the system. The q_{max} value of these isotherm models 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. Adsorption isotherm shows the distribution of solute between the liquid and solid phases and can be described by the standard Langmuir isotherm (Figure 6 and Table 5) and Freundlich isotherm model (Figure 7and Table 6). The linearized Langmuir and Freundlich adsorption isotherm parameters showed the value of linear regression coefficients. The coefficients of determination (R^2) are 0.9730 for Langmuir model and 0.9998 for Freundlich isotherm model and the values for linear regression (R^2) indicated that the adsorption nature is well fitted with both models.

Table 5 Langmuir Isotherm Data for the Biosorption of Cr^{6+} ion by *Paecilomyces* sp.

C_e (mmol L^{-1})	q_e (mmol g^{-1})	C_e/q_e (g L^{-1})
6.02	19.86	0.30
6.04	9.87	0.61
6.05	6.57	0.92
6.06	4.92	1.23
6.07	3.92	1.54

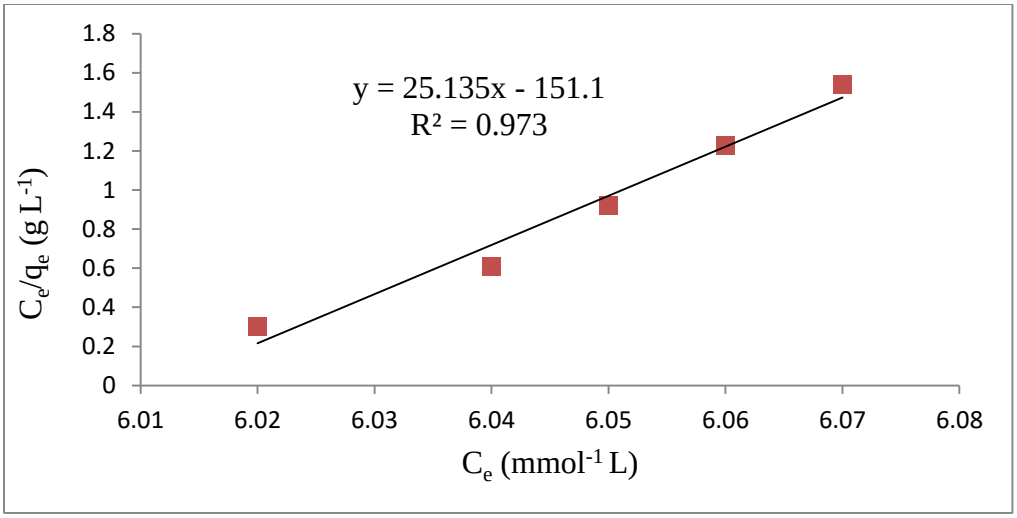


Figure 6 Langmuir adsorption isotherm of Cr^{6+} ion by *Paecilomyces* sp.

Table 6 Freundlich Isotherm Data for the Biosorption of Cr^{6+} ion by *Paecilomyces* sp.

C_e (mmol L^{-1})	q_e (mmol g^{-1})	log C_e (mmol L^{-1})	log q_e (mmol g^{-1})
6.0274	19.8629	0.7801	- 0.10783
6.0480	9.8798	0.7816	- 0.10701
6.0563	6.5728	0.7822	- 0.10668
6.0635	4.9205	0.7827	- 0.10639
6.0738	3.9261	0.7835	- 0.10598

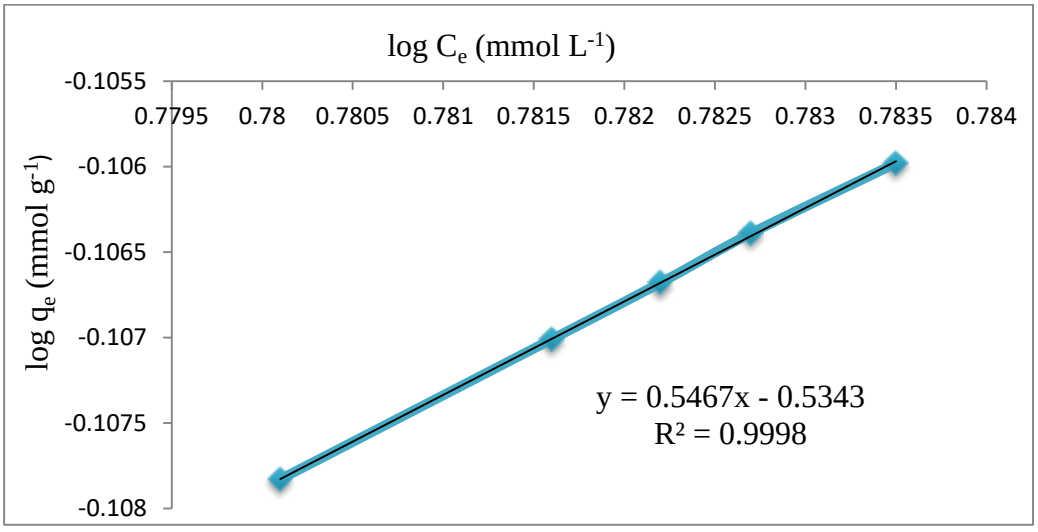


Figure 7 Freundlich adsorption isotherm of Cr^{6+} ion by *Paecilomyces* sp.

Table 7 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
Cr^{6+}	*Paecilomyces* sp.	1.9058	0.9730	0.0036	0.3137	0.9998	1.9632

Conclusion

Soil fungi was isolated from agriculture soil samples by using conventional plate method. Morphological and microscopic characters of the isolated fungi were identified by using keys of Ando (2016). According to morphology and distinct characters, chromium- resistant fungus M-1 was identified as *Paecilomyces* sp. Fungal isolate was tested for their tolerance against different concentrations of Cr^{6+} metal ion. In PDB liquid medium, isolate exhibited fair tolerance towards high chromium concentration. The results suggested that biomass production was higher in control and lower in Cr^{6+} metal ion treated media. In solid PDA medium, the metal concentration reached 50 mM, the fungas failed to grow after culturing for 15 days and 50 mM was thought as the MIC of chromium ion. Thus, it can be concluded both media have similar potential to tolerate various concentration of Cr^{6+} ion. In the biosorption study of wastewater treatment, the industrial wastewater was collected and initial metal ion concentrations of chromium in collected sample was analyzed. According to results, ability of *Paecilomyces* sp. was found to adsorb the chromium ion with maximum removal of 45.48 % at optimum dose 0.4 g and contact time 8 h.

The results of this study revealed that the fungal cell of *Paecilomyces* sp. has greater potential application for the removal of chromium ion from industrial wastewater sample.

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