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Tolerance and biosorption of copper (Cu) and lead (Pb) by filamentous fungi isolated from a freshwater ecosystem

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Abstract

Filamentous fungi are able to accumulate significant amount of metals from their environment. The potential of fungal biomass as agents for biosorption of heavy metals from contaminated sediments is currently receiving attention. In the present study, a total of 41 isolates of filamentous fungi obtained from the sediment of the Langat River, Selangor, Malaysia were screened for their tolerance and uptake capability of copper (Cu) and lead (Pb). The isolates were identified as *Aspergillus niger*, *A. fumigatus, Trichoderma asperellum, Penicillium simplicissimum* and *P. janthinellum*. *A. niger* and *P. simplicissimum*, were able to survive at 1000 mg/L of Cu(II) concentration on Potato Dextrose Agar (PDA) while for Pb, only *A. niger* survived at 5000 mg/L concentration. The results showed that *A. niger*, *P. simplicissimum* and *T. asperellum* have a better uptake capacity for Pb compared to Cu and the findings indicated promising biosorption of Cu and Pb by these filamentous fungi from aqueous solution. The present study was also determined the maximum removal of Cu(II) and Pb(II) that was performed by *A. niger*. The metal removal which occurred at Cu(II) 200 mg/L was (20.910 ± 0.581) mg/g and at 250 mg/L of Pb(II) was (54.046 ± 0.328) mg/g.

Key words: biosorption; copper; lead; *Aspergillus niger*; *Penicillium simplicissimum*; *Trichoderma asperellum*

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Introduction

Heavy metal release into the environment found increased continuously as an impact from industrial activities and technological development. It poses a significant threat to the environment, public and soil health (Zafar et al., 2007). Due to their application and immutable nature increased recently, heavy metals pollution has naturally become one of the most serious environmental problems.

Heavy metals including copper (Cu) and lead (Pb) are continuously being released into the environment as a result of industrial activities and technological development. Industrial effluents such as those produced by mining and metal processing commonly contributed to heavy metal pollution (Savvaidis et al., 2003; Anand et al., 2006). Conventional method for removing metal ions from aqueous solution has been studied in detail such as chemical precipitation, ion exchange, electrochemical treatment and membrane technologies. However, these methods are ineffective and uneconomical (Wang and Chen, 2006). Therefore, rapid, economical and environmentally benign technologies are needed to develop for removing heavy metals from industrial effluents.

Biosorption is a term which involves the use of microbes to detoxify and control environmental contaminants. Recently, it has received increasing attention to clean up polluted sites (Farhadian et al., 2008). By using nonviable microbial biomass including algae, fungi and bacteria, this alternative method has been considered the preferred approach for removing heavy metals and radionuclides from the aqueous solution (Sar and D'Souza, 2001). Biosorption, based on the interactions between living and non-living microorganisms and metallic ions in the system, offers advantages such as low operating cost and high efficiency for removing low concentrations of heavy metal from wastewater (Kapoor et al., 1999; Fan et al., 2008).

Fungi are a versatile biosorption group as they can grow under extreme conditions of pH, temperature and nutrient availability as well as high metal concentration (Anand et al., 2006). Among other biosorption agent, fungi biomasses have a high percentage of cell wall material that shows excellent metal-binding properties. These microorganisms have been employed in the remediation of wastes and wastewaters (Singh, 2006). There is growing interest in

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the application of fungal biomass for bioremediation and a broad range of results have been reported (Lopez and Vazquez, 2003).

In fermentation and bioremediation industries, filamentous fungi have been widely used for decades (Luef et al., 1991). They are preferred over other organisms for bioremediation because they are easier to remove from liquid substrates. Fungal biomass maybe utilized by biosorptive processes as it often exhibits marked tolerance towards metals and other factors such as low pH (Gadd, 1990; Zafar et al., 2007; Kapoor and Viraraghavan, 1997).

In the concept of biosorption, several chemical processes may be involved, such as adsorption, ion exchange and covalent bonding with the biosorptive sites of the microorganisms including carboxyl, hydroxyl, sulfhydryl, amino and phosphate groups (Frurest and Volesky, 1997; Say et al., 2001). Fungal cell walls and their components have a major role in biosorption. Many fungal species such as *Rhizopus arrhizus*, *Penicillium spinulum* and *Aspergillus niger* have been extensively studied for heavy metal biosorption and the mechanism seems to be species dependent (Zhou and Kiff, 1991; Hafez et al., 1997).

In this study, the ability of filamentous fungi isolated from the sediment of the Langat River, Selangor, Malaysia was screened for their tolerance and uptake capacity for different concentrations of Cu(II) and Pb(II). The maximum biosorption capacity based on dry weight was determined by varying the concentrations of Cu and Pb ions in the aqueous solution.

1 Materials and methods

1.1 Sampling

An open water system at the Langat River, Selangor, Malaysia into which municipal and industrial waste flowed was selected as the sampling area. Sediment from this sampling site was collected at 5 to 10 cm deep using an Ekman-Grab sampler (Model 196 Standard Ekman Grab, Wildlife Supply, USA). The sediment samples were put in acid-washed polythene bags and were kept in a refrigerator at 3°C.

1.2 Fungal isolation

A stock solution was made by adding 10 g of sediment with 100 mL of steriled distilled water in a conical flask. The solution was placed on an orbital shaker (Model Orbitron, Infors-HT, Switzerland) at 200 r/min for 20 min. Ten-fold dilution was prepared and the solution at 10^{-3} was used for fungal isolation. Soil solution of 1 mL was pippetted and plated into each Petri dish followed by pouring 9 mL of sterilized rose bengal agar (RBA). The Petri dish was swirled manually before solidification and incubated for four days at (28 ± 2)°C. All colonies that appeared on each plate were marked, the colony forming unit (CFU) was calculated and subcultured onto potato dextrose agar (PDA). Pure cultures were maintained on PDA slants and kept at 4°C until required for further studies.

1.3 Identification of fungi by morphological characteristics and internal transcribed spacer (ITS) sequences

All the fungal isolates were initially identified based on their macroscopic and microscopic characteristics. *Aspergillus* species were identified following Raper and Fennell (1965) and *Trichoderma* species according to Rifai (1969). *Penicillium* species were identified according to Raper et al. (1968).

Fungal identification was confirmed by molecular approaches based on the gene sequencing of the ITS regions of the ribosomal DNA (rDNA). The ITS 1 and ITS 2 regions of the rDNA were amplified by using polymerase chain reaction (PCR) according to White et al. (1990). The multiple sequence alignments were developed from the sequence data and ITS sequences of 10 fungal isolates were compared with sequences from the GeneBank (National Centre for Biotechnology Information; http://www.ncbi.nlm.nih.gov) through BLAST searches.

1.4 Screening of the most tolerant fungi

The Cu(II) and Pb(II) stock solutions were prepared by dissolving $CuCl_2 \cdot 5H_2O$ or $Pb(NO_3)_2$ (analytical grade, Merck) into distilled water. Solid medium was prepared by pouring 2 mL of stock solution of Cu(II) or Pb(II) followed by 18 mL of sterilized PDA into a universal bottle to obtain the desired Cu(II) concentrations of 100, 500, 800 and 1000 mg/L and Pb(II) concentrations of 100, 500, 1000, 2000 and 5000 mg/L. The universal bottle then was shaken to homogenize the solution. The PDA incorporated with Cu(II) and Pb(II) solutions were then immediately poured into the Petri dishes and were swirled gently. The medium was inoculated with one disc (5 mm diameter of cork borer) of young mycelium from the edge of the stock fungus culture (Zapotoczny et al., 2006) and were observed daily for 10 days. The screening was conducted in three replicates for each isolate.

1.5 Toxicity test of selected species

Potato dextrose broth (PDB) was prepared and stock solution of either Cu(II) or Pb(II) was added to the medium in each 250 mL conical flask separately to reach the required concentrations (10, 25, 50, 100, 150, 200, 250 and 300 mg/L) in a volume of 100 mL. The medium was inoculated with three disks of young mycelia from the edge of the stock solid PDA cultures (Zapotoczny et al., 2006). Initial concentrations of Cu(II) and Pb(II) in each conical flask were checked by using an Inductive Coupled Plasma Spectrometer (ICP-OES Model Optima 2000DV, PerkinElmer, USA) before fungal inoculation (Lopez and Vazquez, 2003). Cultures were incubated at (28 ± 1)°C with 200 r/min in an incubator shaker (Model Multitron, Infors-HT, Switzerland).

1.6 Determination of fungal growth at different concentrations of Cu(II) and Pb(II)

After seven days of incubation, flasks containing fungal

biomass at different concentrations of Cu(II) or Pb(II) were harvested and filtered through Whatman No. 1 filter paper. Biomass samples were rinsed several times with distilled water and left in an oven at 80°C until a constant weight was achieved and defined as dry biomass (g/L) (Anand et al., 2006).

1.7 Removal of Cu and Pb at different concentrations by the filamentous fungi

According to method of Lopez and Vazquez (2003), concentrations of Cu(II) or Pb(II) in liquid cultures were measured with ICP-OES before fungal inoculation. The amount of heavy metal uptake (q, mg/g) was calculated by using the following equation (Lopez and Vazquez, 2003; Zafar et al., 2007; Fan et al., 2008):

$$q = [(C_i - C_f)/m]V$$

where, q (mg/g) is mg of metal ions uptake per gram biomass; C_i (mg/L) is the initial metal concentration; C_f (mg/L) is the final metal concentration; m (g) is the amount of dry biomass; and V (L) is the volume of the medium.

1.8 Data analysis

Statistical analyses of data were performed by using the Statistical Package for the Social Science (SPSS), version 16.0. One way analysis of variance (ANOVA) was carried out to show the significance difference at $p \leq 0.05$. Duncan post hoc test was used to compare the means.

2 Results and discussion

2.1 Identification of fungi based on morphological characteristics and ITS sequences

A total of 41 isolates of filamentous fungi were obtained from the sediment of the Langat River, Selangor, Malaysia which had high concentration of Cu (44.43 μg/g) and Pb (17.28 μg/g). The fungal isolates were classified into three genera, i.e., *Trichoderma* species, *Aspergillus* species and *Penicillium* species. All isolates obtained were identified based on their morphological characteristics and reconfirmation by ITS sequences of the isolate. The fungal isolates were identified as *A. niger* (9 isolates), *A. fumigatus* (6 isolates), *T. asperellum* (9 isolates), *P. simplicissimum* (10 isolates) and *P. janthinellum* (7 isolates).

From the 41 isolates, 10 isolates (two isolates from each species which randomly picked) were chosen for species reconfirmation by using ITS sequence. They were successfully amplified by ITS 1 and ITS 4 primers and produced single pattern band around 524 to 626 bp. The BLAST result through the GeneBank showed the regions of local similarity between sequences (Table 1).

2.2 Screening for the most tolerant fungi

According to Zapotoczny et al. (2006), solid medium was more appropriate for the screening study despite the fact that agar may affect metal speciation and solubility. Exposure of soil fungi to heavy metals can lead to physiological adaptation or the selection of mutants (Gadd, 1993; Zafar et al., 2007). In the present study, all 41 fungi isolates were screened and observed under 100, 500, 800 and 1000 mg/L of Cu(II) and 100, 500, 1000, 2000 and 5000 mg/L of Pb(II) on PDA within 10 days of incubation. The results showed that only *A. niger* and *P. simplicissismum* isolates tolerated and showed mycelium growth at 1000 mg/L concentration of Cu(II) while at 5000 mg/L of Pb(II), only the isolate of *A. niger* survived (Table 2).

The growth rates of *A. niger* and *P. simplicissismum* at 1000 mg/L of Cu(II) concentration were significantly different (Table 2). The high tolerance to Cu observed in *A. niger* could be attributed to the fact that the fungus was isolated from a sediment sample containing high levels of metals (Cu: 44.43 μg/g; Pb: 17.28 μg/g). It is known that microorganisms isolated from natural environments contaminated with heavy metals often exhibit tolerance to multiple pollutants because they have adapted to such

Table 1 Closest matches from BLAST searches of fungal sequences

No. of isolates	Closest species match (accession code)	Sequence identity (%)	Sequence overlap (bp)	References
LA01	*Aspergillus niger* (FJ668837.1)[a]	98	625	Huitron et al., 2008
	Aspergillus niger (AY373852.1)	99	623	Haugland et al., 2004
LA09	*Aspergillus niger* (AY213632.1)	99	599	Rakeman et al., 2005
	Aspergillus niger (AY656630.1)	99	622	Bau et al., 2005
LA18	*Aspergillus fumigatus* (AY939804.1)	99	598	Leinberger et al., 2005
	Aspergillus fumigatus (AY373851.1)	98	621	Haugland et al., 2004
LA20	*Aspergillus fumigatus* (GU594756.1)	99	598	Alhanout et al., 2010
	Aspergillus fumigatus (GU566242.1)	99	635	Bukovska et al., 2010
LT89	*Trichoderma asperellum* (EU280109.1)	100	627	Hoyos-Carvajal et al., 2009
	Trichoderma asperellum (GU198303.1)	99	599	Samuels et al., 2010
LT82	*Trichoderma asperellum* (DQ109538.1)	100	576	Samuels et al., 2006
	Trichoderma asperellum (EU264001.1)	99	573	Hanada et al., 2008
LP33	*Penicillium simplicissimum* (GU981588.1)	98	547	Houbraken et al., 2010
	Penicillium simplicissimum (AF203084.1)	98	549	Tuthill et al., 2001
LP42	*Penicillium simplicissimum* (GU981587.1)	95	547	Houbraken et al., 2010
	Penicillium simplicissimum (DQ026013.1)	97	554	Moreno-Velazquez et al., 2007
LP43	*Penicillium janthinellum* (AJ608945.1)	91	589	Sabev et al., 2006
	Penicillium janthinellum (GQ241286.1)	91	569	Kim et al., 2010
LP48	*Penicillium janthinellum* (DQ682591.1)	91	513	Posada et al., 2007
	Penicillium janthinellum (AY373921.1)	91	612	Haugland et al., 2004

[a] Included are the sequence identities with corresponding Genebank references sequences.

Table 2 Growth rate of fungi on PDA at different concentrations of Cu(II) and Pb(II)

Species	*n*	Control	Cu(II) concentration			
			100 mg/L	500 mg/L	800 mg/L	1000 mg/L
A. niger	27	3.370 ± 0.061 b	3.185 ± 0.036 b	2.963 ± 0.049 b	2.659 ± 0.050 a	1.226 ± 0.045 a
A. fumigatus	18	2.733 ± 0.049 c	1.278 ± 0.043 e	0.156 ± 0.051 e	0.000 ± 0.000 d	0.000 ± 0.000 c
P. simplicissimum	30	1.857 ± 0.051 e	1.483 ± 0.038 c	1.057 ± 0.050 c	0.357 ± 0.050 b	0.147 ± 0.051 b
P. janthinellum	21	2.671 ± 0.046 d	1.381 ± 0.040 d	0.762 ± 0.049 d	0.000 ± 0.000 d	0.000 ± 0.000 c
T. asperellum	27	4.429 ± 0.054 a	4.278 ± 0.042 a	3.259 ± 0.050 a	0.159 ± 0.050 c	0.000 ± 0.000 c

Species	*n*	Control	Pb (II) concentration				
			100 mg/L	500 mg/L	1000 mg/L	2000 mg/L	5000 mg/L
A. niger	27	3.370 ± 0.061 b	3.241 ± 0.057 b	2.926 ± 0.071 b	2.667 ± 0.055 a	2.422 ± 0.085 a	2.048 ± 0.075 a
A. fumigatus	18	2.733 ± 0.049 c	2.550 ± 0.515 c	1.461 ± 0.069 c	0.978 ± 0.073 c	0.233 ± 0.049 c	0.000 ± 0.000 b
P. simplicissimum	30	1.857 ± 0.051 e	1.757 ± 0.063 d	1.137 ± 0.067 d	0.800 ± 0.069 e	0.280 ± 0.061 b	0.000 ± 0.000 b
P. janthinellum	21	2.671 ± 0.046 d	1.676 ± 0.044 e	1.181 ± 0.060 d	0.848 ± 0.051 d	0.241 ± 0.066 c	0.000 ± 0.000 b
T. asperellum	27	4.429 ± 0.054 a	4.189 ± 0.058 a	3.000 ± 0.198 a	1.189 ± 0.064 b	0.285 ± 0.082 b	0.000 ± 0.000 b

n is the number of fungal isolates performed in triplicate. The growth rate of fungi were compared by using one-way ANOVA and Duncan's Post Hoc Test for different concentrations of Cu(II). Means with different letters in each column of Cu(II) and Pb(II) concentrations were significantly different at $p \leqslant 0.05$.

environments. Price et al. (2001) reported that *A. niger* grew better in the presence of Cu than *P. simplicissimum*, *F. verticillioides*, *Rhizoctonia solani*, *Aquathanatephorus pendulus* and *Geotrichum* species.

In the present study, the fungal mycelia gave a deep blue appearance at 500, 800 and 1000 mg/L of Cu(II) concentrations. This result is similar with those of Venkateswerlu et al. (1989) who determined that there were blue colored in the presence of Cu in *Neurospora crassa* and *Cunninghamella blackesleeana* and they suggested this was caused by the binding of the Cu ions to the protein in the cell wall of mycelium. Zapotoczny et al. (2006) also reported that *Acremonium pinkertoniae* could tolerate up to 600 mg/L concentration of Cu on malt agar.

T. viride growth was tested for Cu ranged from 1000 to 5000 mg/L on agar media and it was found that 5000 mg/L was the maximum tolerance of this species (Anand et al., 2006). Variation in metal tolerance might be due to the presence of one or more types of tolerance strategies or resistance mechanisms exhibited by different fungi (Zafar et al., 2007). *A. niger*, *P. simplicissimum* and *T. asperellum* were later selected for use in Cu and Pb removal capability studies based on the previous statistical analysis. These three fungal species were determined as being the most tolerant fungi to Cu(II) and Pb(II) when compared to the other species. Next, the removal of Cu(II) and Pb(II) by *A. niger*, *P. simplicissimum* and *T. asperellum* were determined in the PDB at different Cu(II) and Pb(II) concentrations.

2.3 Cu(II) uptake capacity by selected filamentous fungi

Dry biomass of *A. niger*, *P. simplicissimum* and *T. asperellum* decreased with increasing initial concentrations of Cu(II) (Fig. 1). The result indicated that the highest biomass values of those fungi were at the control concentration while the lowest biomass values was recorded at 300 mg/L of Cu(II). It was found that at 200 mg/L of Cu(II), the maximum Cu uptake occurred and the highest dry biomass was obtained by *P. simplicissimum* followed by *T. asperellum* and *A. niger.* The lowest dry biomass of *A. niger* when compared to the fungi however gave the highest Cu uptake capacity. In a previous study, Lopez and Vazquez (2003) reported that *T. atroviride* survived at concentrations of 0 to 300 mg/L but dramatically decreased in growth at 350 mg/L of Cu. Tsekova and Todorova (2002) also reported a similar level of tolerance of Cu(II) by *A. niger* strain B-77 and the inhibitory concentration of Cu(II) for this strain was 300 mg/L. In addition, Anand et al. (2006) reported there was no growth of *T. viride* at 300 mg/L of Cu(II).

With increasing the initial concentration of Cu(II) up to 200 mg/L in the culture medium, uptake capacity by *A. niger*, *P. simplicissimum* and *T. asperellum* were also increased (Fig. 2). Maximum uptake of Cu from the metal solution occurred at 200 mg/L concentration with a value of 20.910 mg/g by *A. niger*, 16.179 mg/g by *P. simplicissimum* and 12.809 mg/g by *T. asperellum*. From 10 to 200 mg/L of Cu concentration, uptake slowly increased and then began to decrease at 250 mg/L of Cu(II). In the present study, Cu(II) uptake was increased with increasing Cu(II) initial concentration but it became saturated and precipitated at 200 mg/L.

According to Deshmokh and Rai (2005), the uptake increase may often be associated with toxicity or increasing permeabilization of cell membrane on account of further binding of the metal to exposed intracellular sites. Metal-

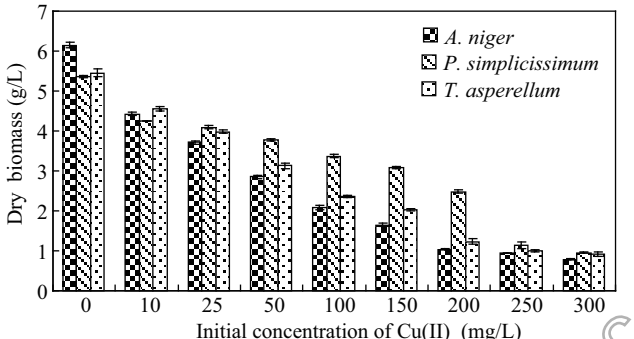

Fig. 1 Dry biomass of *A. niger*, *P. simplicissimum* and *T. asperellum* fungi in liquid medium at different concentrations of Cu(II).

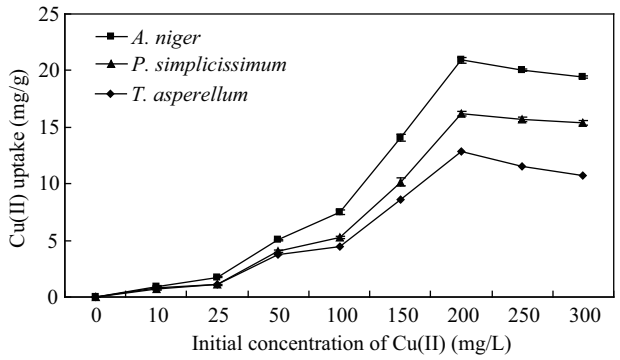

Fig. 2 Uptake capacity of Cu(II) at different concentration of Cu(II) by *A. niger*, *P. simplicissimum* and *T. asperellum*.

lothioneins (MTs) are metal binding proteins which have been postulated as being responsible for detoxification of a variety of class IIb metals in many different species (Lerch, 1980; Jeyaprakash et al., 1991; Price et al., 2001). *A. niger* is known to possess a copper metallothionein (CuMT), but neither the protein nor the gene has been sequenced (Kermasha et al., 1993). Akhtar and Mohan (1995) examined the ability of *A. niger* to remove the contaminating metals. They reported that the chemically treated *A. niger* was able to remove 17.02 μg Cu/g fungal dried weight from the contaminated lake water.

2.4 Pb(II) uptake capacity by filamentous fungi in liquid medium

Growth of *A. niger* was higher compared to *P. simplicissimum* and *T. asperellum* at all concentrations of Pb(II) except at 50 mg/L (Fig. 3). Increasing Pb(II) concentration may lead to the decrease of microbial growth. At 250 mg/L, where the highest Pb(II) uptake occurred, the dry biomass obtained for *A. niger* was 2.908 g/L and followed by *T. asperellum* (2.738 g/L) and 1.353 g/L for *P. simplicissimum*.

The growth and metal removal properties of the three fungi were highly affected by initial metal ion concentration. The highest Pb(II) uptake (54.047 mg/g) at 250 mg/L initial Pb(II) concentration was obtained for *A. niger*, whereas *P. simplicissimum* and *T. asperellum* were only able to remove 38.975 mg/g and 17.619 mg/g Pb(II), respectively (Fig. 4). Fan et al. (2008) reported that the maximum Pb(II) ion uptake capacity by *P. simplicissimum* was 76.90 mg/g at 250 mg/L of Pb(II) concentration.

Uptake capacities were decreased at 300 mg/L as at this concentration the aqueous solution reached it saturation stage. Say et al. (2001) also reported that the saturation value was around 300 mg/L for all heavy metals. They reported that the fungal cell walls have a negative charge due to the arrangement of the carboxyl and phosphate groups of the cell walls. Based on the investigation of Pb(II) uptake capacity by *A. niger*, Kapoor and Viraraghavan (1997) revealed that biosorption of heavy metal by fungi occurs as a result of ionic interaction and complex formation between metal ions and functional group present on the fungal cell surface. Akhtar et al. (1996) suggested the functional groups which may be involved in the biosorption of heavy metals include phosphate, carboxyl, amine and amide groups.

The uptake capacity of Cu(II) and Pb(II) by *A. niger* was found to be the highest when compared to *P. simplicissimum* and *T. asperellum*. It was also shown that the three fungi tested above had removed more Pb(II) ion compared to Cu(II) (Table 3). This result is in agreement to those of Akar and Tunali (2006), who reported that the order of affinity for noncompetitive condition (based on mg/g accumulation) was: Pb(II) > Cu(II) by *A. flavus*. They also found that Cu(II) and Pb(II) removal by *A. flavus* was increased with increasing initial metal concentration. Yalcin et al. (2010) suggested that a higher initial concentration provided an important driving force to overcome all mass transfer resistances between the metal solution and the fungi cell wall. Aksu and Tezer (2005) also reported that the number of collisions between metal ions and a biosorbent increases with increasing initial metal concentrations and thus, the biosorption is enhanced.

However, at certain higher concentration of metal, the biosorption efficiency decreased. The present study indicated that at 200 mg/L (20.910 ± 0.581 mg/g) and 250 mg/L (54.046 ± 0.328 mg/g) of Cu(II) and Pb(II) concentrations respectively, the solution became saturated and metal precipitation occurred. The number of metal ions competing for the available binding sites in the biomass and the lack of binding sites for the complexion of Cu(II) and Pb(II) has caused the solution to become into a saturated stage (Akar and Tunali, 2006).

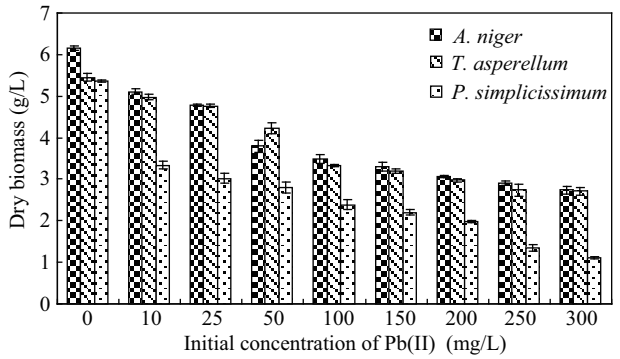

Fig. 3 Dry biomass of *A. niger*, *P. simplicissimum* and *T. asperellum* in liquid medium at different concentrations of Pb(II).

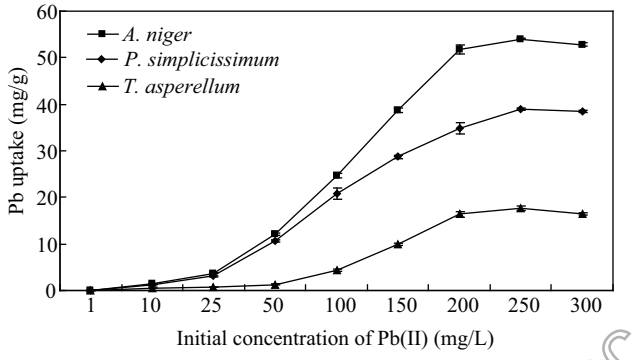

Fig. 4 Uptake capacity of Pb(II) by *A. niger*, *P. simplicissimum* and *T. asperellum* at different concentration of Pb(II).

Table 3 Comparison of uptake capacity for Cu(II) and Pb(II) by *A. niger*, *P. simplicissimum* and *T. asperellum* at different concentrations

Fungi species	Initial (mg/L)	Uptake capacity (mg/g)	
		Cu(II)	Pb(II)
A. niger	0	–	–
T. asperellum		–	–
P. simplicissimum		–	–
A. niger	10	0.959 ± 0.021 a	1.511 ± 0.045 a
T. asperellum		0.659 ± 0.027 b	1.266 ± 0.103 b
P. simplicissimum		0.801 ± 0.017 c	0.598 ± 0.114 c
A. niger	25	1.754 ± 0.059 a	3.738 ± 0.102 a
T. asperellum		1.105 ± 0.106 b	3.092 ± 0.121 b
P. simplicissimum		1.069 ± 0.123 b	0.733 ± 0.108 c
A. niger	50	5.022 ± 0.072 a	12.099 ± 0.682 a
T. asperellum		4.026 ± 0.162 b	10.739 ± 0.245 b
P. simplicissimum		3.790 ± 0.144 c	1.318 ± 0.311c
A. niger	100	7.495 ± 0.395 a	24.701 ± 0.819 a
T. asperellum		5.234 ± 0.211 b	20.831 ± 2.338 b
P. simplicissimum		4.491 ± 0.189 c	4.309 ± 0.418 c
A. niger	150	14.042 ± 0.625 a	38.658 ± 1.053 a
T. asperellum		10.161 ± 0.736 b	28.741 ± 0.679 b
P. simplicissimum		8.615 ± 0.256 c	9.960 ± 0.569 c
A. niger	200	20.910 ± 0.581 a	51.804 ± 1.909 a
T. asperellum		16.179 ± 0.385 b	34.798 ± 0.236 b
P. simplicissimum		12.809 ± 0.274 c	16.393 ± 0.656 c
A. niger	250	20.033 ± 0.194 a	54.046 ± 0.328 a
T. asperellum		15.676 ± 0.358 b	38.579 ± 0.581 b
P. simplicissimum		11.558 ± 0.311 c	17.619 ± 0.426 c
A. niger	300	19.439 ± 0.113 a	52.819 ± 0.873 a
T. asperellum		15.374 ± 0.370 b	38.387 ± 0.495 b
P. simplicissimum		10.767 ± 0.416 c	16.499 ± 0.579 c

* Mean ± SD (n=4) with different letters showed that at different concentration, there were a significant different in Cu(II) and Pb(II) uptake capacity among the three fungi. The uptake capacity by fungi were compared by using one-way ANOVA and Duncan's Post Hoc Test at different concentration of Cu(II) and Pb(II).

3 Conclusions

The present study indicated the potential of *A. niger*, *P. simplicissimum* and *T. asperellum* to become good biosorbent agents for Cu(II) and Pb(II). These fungi were shown to have a better uptake capacity for Pb(II) compared to Cu(II) and the uptake capacity found increased as initial metal concentration increased. However, the efficiency of metal uptake decreased when the medium achieved a saturated stage.

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