

Full Length Research Paper

# Bioleaching of copper, aluminum, magnesium and manganese from brown shale by *Ganoderma lucidum*

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Accepted 27 July, 2011

The present study was done to check the bioleaching feasibility of brown shale for the recovery of copper (Cu), aluminum (Al), magnesium (Mg) and manganese (Mn) ions using *Ganoderma lucidum*. Different experimental parameters were optimized for the enhanced recovery of metals ions. Effect of different substrates like glucose, molasses, saw dust and cotton seed cake on the recovery of metals ions was investigated under shaking as well as non-shaking conditions. Significant difference in leaching of metal ions by *G. lucidum* was observed under shaking and non shaking conditions. Maximum leaching of Al (90.7%), Mg (96.46%), Mn (66.3%) and Cu (73.45%) was observed using glucose under shaking conditions with 5, 3, 4 and 3% pulp densities respectively. The results show that maximum solubilization up to 68.89, 77.03 and 38.37% was achieved for Cu, Al and Mg ions respectively using molasses as substrate, whereas, 57.74% recovery of Mn was achieved with saw dust. The recovery of metal ions indicated that this low grade discarded ore may be a potential source of metal ions in future.

**Key words:** Recovery, brown shale, *Ganoderma lucidum*, organic acids, pulp density.

## INTRODUCTION

In current scenario, world demand for metals is growing fast from low grade ores and wastes due to depletion of high grade ores. Minerals industry is increasingly faced with the need for economic extraction of metal ions from low-grade ores. As millions of tonnes of low-grade ore and copper-rich tailings await the development of an efficient and economic extraction process, the recovery of metal ions from low and lean grade ores, such as shales and schist, by conventional techniques is very expensive due to high energy and capital inputs. Moreover, the conventional technologies are associated with environmental hazards. As environmental standards regarding toxic wastes continue to establish, the costs for ensuring environmental protection continue to rise (Devasia and Natarajan, 2004; Pradhan et al., 2006; Anjum et al., 2009).

Microbial technology offers an economic alternative for the mining industry, at a time when high grade mineral resources are being depleted (Rawlings, 2004). Generally, bioleaching refers to the conversion of metals into their

water soluble forms by microorganisms (Rawlings et al., 2003; Olson et al., 2003; Ndlovu, 2008). Brown shales undergo chemical leaching and the metal dissolution processes are also mediated by microorganisms. Microorganisms like heterotrophs require carbon as an energy source, and this requirement can be fulfilled using organic wastes. Acidolysis, redoxolysis and complexolysis are the principal mechanisms in bioleaching of metals by fungi. The fungus produces organic acids such as citric, oxalic, malic and gluconic acids during bioleaching (Mulligan et al., 2004; Johnson, 2006). Most active leaching fungi which have been isolated and used are from the genera *Penicillium* and *Aspergillus* (Burgstaller and Schinner, 1993). Experiments have shown that *Aspergillus niger* exhibited good potential in generating organic acids (oxalic, citric, malic and tartaric acids) effective for metal solubilization and maximum solubilization of 68, 46 and 34% have been achieved for copper, zinc and nickel, respectively (Catherine et al., 2004). Whereas *Penicillium simplicissimum* has been widely used for bioleaching of valuable elements (Al, Co, Cu, Ni) from ores (Sukla, 1993) and *Penicillium citrinum* has been used for 64.6% removal of Mn from low grade magniferous ores (Acharya et al., 2004).

*G. lucidum* is a basidiomycete belonging to Poly-

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Poraceae also known as “Ling Zhi” in china and “Reishi” in Japan. Because of its high medicinal value, *G. lucidum* has received wide popularity as a health and medicine in the Far East for a long time. No enough attention has been given towards its bioleaching capabilities. In 1965, Takao screened organic acid producing strains of basidiomycetes and found that oxalic acid was the main acid along with citric and tartaric acid. Yang (2005), investigated about the Ganoderic acid production by *G. lucidum*. These acids may be helpful for leaching metals by complexation.

Brown shale is a very fine-grained sedimentary rock which has been formed by the consolidation of beds of mud, clay or silt along with residues of algae, bacteria and other life forms that lived in the sea. These are composed chiefly of clay minerals, quartz, and illite (Bohacs et al., 2000). Pakistan has huge reserves of black and brown shale deposits that is, the Foothills of Himalayas at Lagarban, in Karakoram valley at Shishi Gol and South of Mirkhani, Kakul in Hazara District, the Chitral areas as well as the Turbela areas (Ahmad, 1969).

The main concern of the present research work was to explore the ability of microbes like *G. lucidum* to extract metal ions like Al, Mg, Cu and Mn from brown shale using different experimental strategies and agriculture wastes as substrates.

## MATERIALS AND METHODS

### Brown shale sample

Brown shale ore sample was obtained from Mineralogical Centre Chakwal, Pakisan. It was alkaline (pH 7.8) and insoluble in water. The sample was oven dried and ground to 200 mesh size by ASTM sieving machine. Elemental analysis was carried out by atomic absorption spectrophotometry (Perkin Elmer, A Analyst 300). Raw sample of brown shale mainly composed of (mg/kg) Ca 869, Al 365.4, Mg 247.4, Cu 147, Mn 95.8 and Ni 43.7.

### Fungal strain and growth conditions

*G. lucidum* was procured from Industrial Biotechnology Laboratory (IBL), Department of Chemistry of Biochemistry University of Agriculture Faisalabad Pakistan. It was then propagated and maintained on potato dextrose agar (PDA) slants (3.9%) as described by Bosshard et al. (1996) and stored in an incubator (Incubator, Sanyo, Germany) for 72-96 h at 30°C to produce an adequate growth. For growth in liquid medium, the culture medium consist of (g/L): 0.22; ammonium tartarate, 0.21; KH<sub>2</sub>PO<sub>4</sub>, 0.05; MgSO<sub>4</sub>.7H<sub>2</sub>O, 0.01; CaCl<sub>2</sub>, 0.001; Thiamine, 0.08; CuSO<sub>4</sub>, 0.05; H<sub>2</sub>MoO<sub>4</sub>, 0.07; MnSO<sub>4</sub>.4H<sub>2</sub>O, 0.043; ZnSO<sub>4</sub>.7H<sub>2</sub>O, 0.05; Fe(SO<sub>4</sub>)<sub>3</sub> and then 10 mL of 10% Tween 80, 10 mL of 100 mM veratryl alcohol, 1mL of chloramphenicol was added and volume was made up to 1000 mL with distilled water (Bibi et al., 2009). Four sets of 250-mL flasks were prepared containing 100 mL of liquid medium each in triplicate samples. Medium in each flask was autoclaved at 121°C for 15 min. After sterilization, 5% of given substrate was added in each flask then inoculated with 1 mL of *G. lucidum* spore suspension as inoculum (approximately  $1.9 \times 10^6$  spores/mL). All the flasks were sealed with removable cotton and incubated in an orbital shaker (Gellen Kamp, England) at 30°C at an operating speed of 120

rpm for 15 days.

### Sources and pretreatment of substrates

Glucose (filtered) was used as substrate in medium A. Molasses obtained from local sugar industry was diluted (50% v/v) and after autoclaving was used as substrate in medium B. Sawdust obtained from a local workshop and cotton seed cake from local grocery were ground and oven dried and then used as substrate in medium C and D respectively (Table 1). These agricultural wastes were immersed in sulfuric acid of pH 2 for 24 h. The amount of these substrates was constant in all the flasks.

### Chemical leaching

To investigate metal ions solubilization in correlation with various concentrations of oxalic, tartaric and citric acids (0.01, 0.05, 0.1 and 0.5%), chemical leaching tests were performed. Triplicate samples with different concentration of organic acids (1% pulp density) were subjected to shaking for a period of 15 days. Liquid samples were filtered and analyzed for metal ions dissolved by atomic absorption spectrophotometry (AAS). pH of the liquid samples was monitored after every 48 h of leaching period.

### Leaching of metals by organic acids produced by *G. lucidum*

After incubation period of 15 days, supernatant liquor of microbial culture both from shaking and non-shaking, containing glucose, molasses, saw dust and cotton seed cake (1%) were collected, centrifuged (8000 rpm for 10 min at 15°C) and filtered to remove solids biomass before HPLC analysis for the determination of organic acid metabolites. Supernatants containing organic acid metabolites were used to leach the metal ions from shale residue. Then 1, 2, 3, 4, 5% (w/v) of shale ore samples were added to media of flasks and incubated in an orbital shaker to keep everything in homogeneous slurry form at 30°C and 120 rpm for 36 days. Samples were collected after every two days of leaching period, pH was monitored after every 24 h and supernatant were analyzed for metal ions analysis (Escobal et al., 1996). After centrifugation and filtration, samples were vortexed before HPLC analysis. The mobile phase consisted of 0.25% of acetic acid, was filtered and sonicated to remove if any of the suspended particles. An HPLC (Sykam GmbH, Kleinostheim, Germany) equipped with S-1121 dual piston solvent delivery system and S-3210 UV/VIS diode array detector and software package for data acquisition was used. A 20 µL of filtered sample was injected into an analytical Hypersil (Thermo Hypersil, GmbH, Germany) ODS reverse phase (C) column (250 4.6 mm; 5 µm particle size) fitted with a C guard column. The chromatographic separation was performed by isocratic elution of the mobile phase at a flow rate of 1.0 mL/min at 30°C. Detection was performed at wavelength of 254 nm. Organic acids were identified by comparing the retention times and quantified on the basis of peak area. The peak areas were recorded and calculated by a computer with, chromatography data acquisition and integration software (SRI Instrument, Torrance, California, USA).

### Metal ions analysis

Samples collected on every fifth day of leaching period, were subjected to metal ions analysis by atomic absorption spectrophotometry (Perkin Elmer, Analyst 300). Although, for Al UV/Visible spectrophotometric method devised by Shull and Guthan (1967) was utilized. At the end of leaching period of each medium, residue samples were washed with water three times

**Table 1.** Pretreatment of substrates.

Substrate	Pretreatment
Glucose	Filtration
Molasses(50% v/v)	Autoclaving (120 °C, 5 min)
Saw dust	Grinding/sulfuric acid pH 2
Cotton seed cake	Grinding/sulfuric acid pH 2

and then oven dried. Residues were subjected to wet digestion process by using nitric acid and hydrogen peroxide.

### Statistical analysis

All experiments of the samples before and after leaching were performed in triplicate and the results were reported as mean  $\pm$  SD (Steel et al., 1997).

## RESULTS AND DISCUSSION

Physically, the shale sample appears brown in color, alkaline in nature and insoluble in water. The coloring material in individual shales might be due to carbon, hydrocarbon or finely divided iron sulfide. Mineralogical analysis of brown shale sample contained Monticellite, Mayenite, Calcite, Magnesite, Quartz, Phlogopite and Montmorillonite minerals, while elemental analysis of ore showed the presence of 3.65, 95.8, 146.90, 247.45, 365.35 and 869.00 mg/L of Ni, Mn, Cu, Mg, Al and Ca respectively.

### Chemical leaching

Figure 1A, 1B and 1C depict the results of chemical leaching. In case of copper solubilization, citric acid showed more potential than oxalic acid; although, oxalic acid is stronger than citric acid (Mulligen et al., 2004). Copper solubilized up to 89.20 mg/L with 0.5% citric acid while at the same concentration, oxalic acid showed 72.60 mg/L solubilization. Similar finding has been reported by Mulligen et al. (2004). Tartaric acid solubilized 101.5 mg/L that is 69.09% Cu with 0.5% concentration. This greater recovery of copper might be due to the formation of copper-tartrate complex in the solution and thus, enhanced solubilization of the metal ions (Wong et al., 2003).

Oxalic acid showed maximum affinity for Mg extraction from brown shale that is 148.70 mg/L (60.09%). The values of leached metal by tartaric acid and citric acid were 146.8 and 121.9 mg/L respectively. For Mn solubilization, citric acid showed significant potential and solubilized 52.95% (50.73 mg/L) manganese with 0.5% acid concentration while oxalic acid solubilized 45.21 mg/L manganese with the same acid concentration and 33.71 mg/L of manganese was solubilized with 0.5% concentration

of tartaric acid. These results suggest that optimal metal solubilization require controlled hydrogen ion concentration. Increased H<sup>+</sup> concentration resulted in less effective metal dissolution.

Citric acid was most preferred organic acid for aluminum solubilization (248.2 mg/L) with 0.5% concentration. Citric acid forms multidentate complexes with metals as it is having 3 replaceable H<sup>+</sup> (Tang and Valix, 2006), while oxalic and tartaric acids with almost same efficiency solubilized Al, 236.4 and 216.8 mg/L, with 0.5 % concentration of acids.

### Organic acids produced by *G. lucidum*

Substrates undergo microbial oxidation during growth studies of *G. lucidum* which resulted in the production of organic acids including citric, oxalic, tartaric and malic acids that play a fundamental role in the environmental mobility of metal ions (Sayer and Gadd, 2001; Mulligan et al., 2004; Xu and Ting, 2009). Some of these acids act as complexing agents for metal ions. Citric, oxalic and tartaric acids have chelating properties and have a higher affinity for Al, Mn and Cu (Tang and Valix, 2006; Wong et al., 2003; Wasay et al., 2001). Organic acids produced in all media were collected before leaching (after 15 days of growing) as well as that of sample collected on the 36th day of leaching and analyzed by HPLC (Table 2) to investigate their utilization. The main acid produced by *G. lucidum* was tartaric acid. In case of molasses, the order of production was tartaric acid (70.05 mg/L, > citric acid (7.808 mg/L) > oxalic acid (3.343 mg/L) as can be seen in Table 2. In contrary, saw dust showed the order of tartaric acid (34.02 mg/L) > oxalic acid (7.060 mg/L) > citric acid (0.662 mg/L) whereas oxalic and citric acid production was not detected in cotton seed cake media while tartaric acid concentration was 11.812 mg/L as shown in Table 2. In contrast to our results, Bhatti et al. (2009) reported that *A. niger* produced oxalic and citric acids in the glucose media and Anjum et al. (2009) reported that citric, malic, oxalic and tartaric acids were produced during the growth studies of *P. notatum*, that play fundamental role in the environmental mobility of metal ions. Burgstaller and Schinner (1993) reported citric acid production at pH around 2.5 by *A. niger*. Schimazona (1955) reported low production of oxalic acid, as most of the species of white rot fungi had the ability to decompose oxalic acid due to the presence of oxalic acid decarboxylase in the mycelium which particularly affected decarboxylation of oxalic acid at the acidic pH.

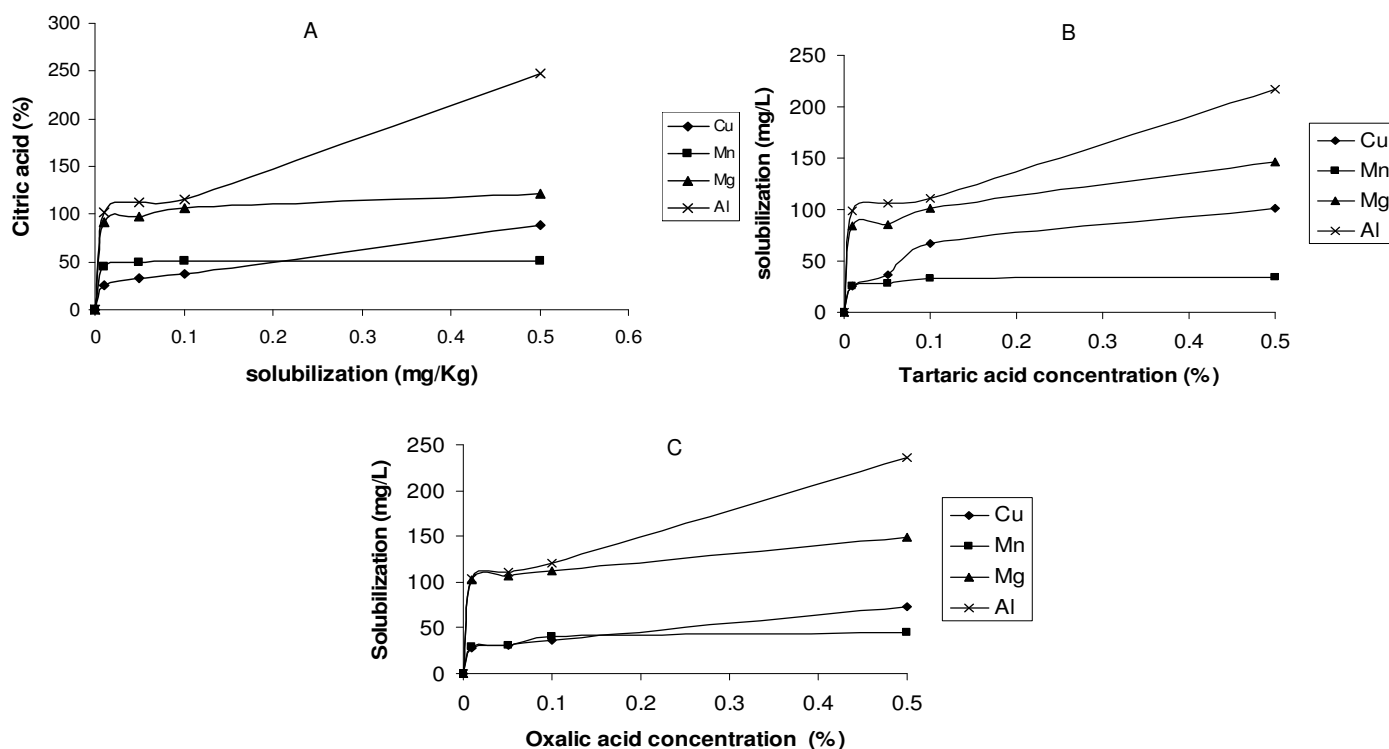
### Leaching of metal ions from brown shale

Bioleaching with fungus *G. lucidum* was carried out to determine if the acids produced by fungus enhance metal leaching in shaken as well as unshaken conditions. Glucose was taken as standard substrate.

**Table 2.** Concentration of organic acids in fermented media after 15 days of fungal growth (before leaching as well as after leaching).

Parameter	Organic acid (mg/L w/v)				
	pH of fermented media	Oxalic acid	Tartaric acid	Citric acid	Malic acid
<b>Medium B (molasses)</b>					
Before leaching	2.9	3.343	70.05	7.808	N.D
After leaching	8.9	N.D	35.828	N.D	N.D
<b>Medium C (saw dust)</b>					
Before leaching	3.0	7.060	34.02	0.662	N.D
After leaching	8.6	N.D	N.D	N.D	N.D
<b>Medium D (cotton seed cake)</b>					
Before leaching	3.1	ND	11.812	ND	ND
After leaching	8.4	ND	ND	ND	ND

ND, Not determined.



**Figure 1.** (A) Citric acid solubilization of metals; (B) Tartaric acid solubilization of metals; (C) Oxalic acid solubilization of metals.

**Copper leaching (constant substrate concentration)**

Copper solubilized up to 73.45% with shaking condition, using glucose (1%) as substrate with 3% of pulp density up to the 36th day of incubation, while maximum recovery of copper has been found in case of molasses (68.89%) up to the 30th day of incubation with 4% pulp density and 1% molasses concentration which is followed by saw

dust (56.67%) and cotton seed cake (50.43%) as shown in Figure 2A. Catherine and Kamali (2003) also reported that molasses although consists of just 30–50% sucrose, showed good potential for solubilization of Cu. Approximately, 48% of the copper had solubilised by day 14th and sawdust was now proved to be a good substrate as it only solubilised only 8% Cu using *A. niger* under shaking conditions, while under unshaken conditions, glucose

conditions, glucose solubilized up to 65.03% of Cu with 3% pulp density on the 36th day of incubation, saw dust showed maximum potential (55.40%) for copper at 4% pulp density at 1% saw dust concentration on the 36th day of incubation, followed by molasses (51.40%) and cotton seed cake (44.67%) as depicted in Figure 2B.

#### **Manganese leaching (constant substrate concentration)**

Manganese solubilization under shaking conditions is shown in Figure 3A. Mn recovery of up to 66.31% was attained after 33 days of incubation and 4% pulp density using glucose (1%) as substrate. In case of saw dust, maximum recovery of 55.20% was attained on the 33rd day of incubation and 4% pulp density whereas cotton seed cake and molasses solubilized only 20.41 and 46.99% of Mn. Figure 3B indicates that under unshaken conditions, glucose (1%) solubilized up to 61.90% of Mn at 3% pulp density and on the 36th day of incubation. Maximum recovery of Mn (57.74%) was achieved with saw dust as substrate and 2% pulp density of ore on the 33rd day of incubation. Molasses recovered 41.56% Mn, while cotton seed cake solubilized 36.70% of Mn. Higher rates of solubilization of Mn might be due to the reason that the *G. lucidum*, a white rot fungus has extracellular lignolytic peroxidases and Mn peroxidases is one of its main enzymes (Galkin et al., 1998).

#### **Magnesium leaching (constant substrate concentration)**

The results relating to Mg solubilization in shaking flasks at constant substrate concentration are shown in Figure 4A which indicated that in shaking conditions, 96.46% Mg was recovered on the 33rd day of incubation and 3% pulp density by using glucose as substrate. While under unshaken conditions, glucose solubilized up to 73.01% of Mg at 4% pulp density on the 36th day of incubation. Whereas taking into account the agricultural wastes, molasses recovered 71.20% of Mg after 30 days of incubation and 4% pulp density under shaking conditions and 77.03% Mg solubilization under unshaken conditions followed by saw dust (65.83%) and cotton seed cake (38.42%) under shaking and 60.57% and 34.09% under unshaken conditions at the 36th day of incubation (Figure 4B). Anjum et al. (2010) also reported that maximum metal leaching was occurred at the 36th day of incubation under shaking conditions.

#### **Aluminum leaching (constant substrate concentration)**

The results representing Al solubilization in shaking

flasks are shown in Figure 5A which indicated that recovery of Al was up to 90.70% after 33 days of incubation and 5% pulp density taking glucose concentration as constant (1%) under shaking conditions. While taking into account molasses, maximum recovery of 38.37% was attained on the 30th day of incubation and 5% pulp density followed by saw dust (29.86%) and cotton seed cake (15.24%). Under unshaken flasks, up to 79.16% of Al was solubilized on the 33rd day of incubation with 5% pulp density while with molasses recovery was 31.55% with 2% pulp density and at the 33rd day of incubation followed by saw dust (25.62%) and cotton seed cake (11.58%) Figure 5B. Maximum leaching of Al was attained after 36 days of incubation both in the case of shaking (76.7%) and non shaking cultures (62.7%) (Anjum et al., 2010).

#### **pH profile of leached suspensions**

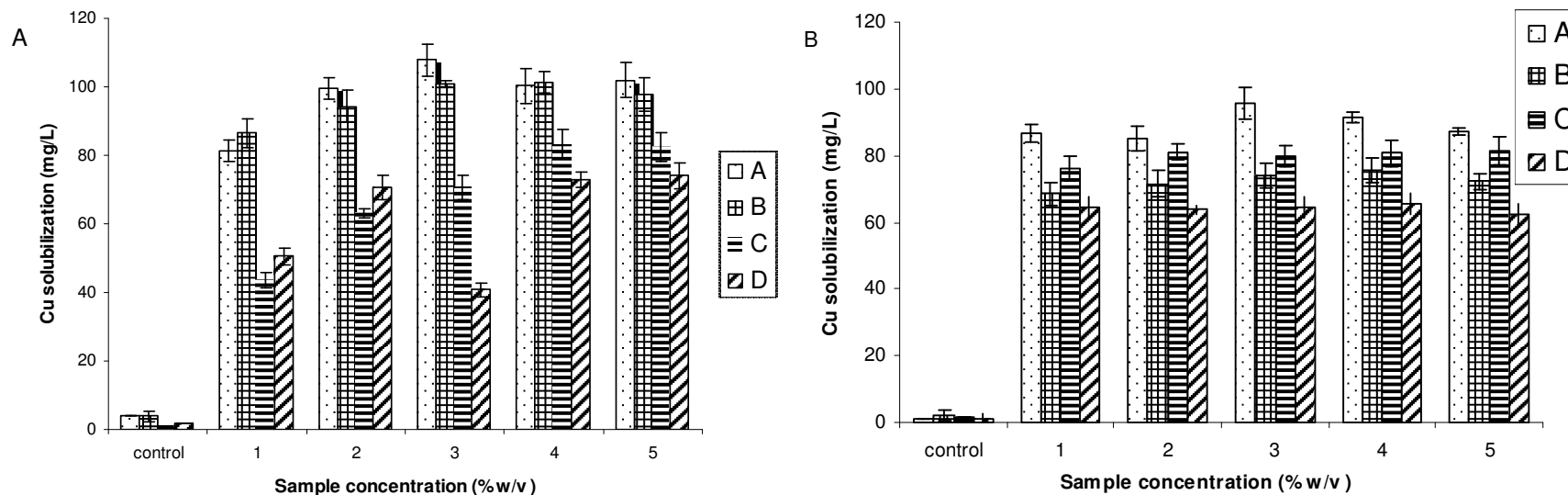
Figures 6 to 9 show the pH profiles of leached suspensions in all the four media that is glucose, molasses, saw dust and cotton seed cake. The initial pH (4.5) of all media inoculated with *G. lucidum* decreased due to the formation of organic acids by the microbial oxidation of carbon source by metabolic activity. The lowest decrease in pH up to 2 units was attained in case of glucose. Similar findings have been reported by Wilscher and Bosecker (2003). According to them, the heterotrophic microorganisms lower the pH of the solution in all leaching experiments by 2 to 4 units. The pH during growth as well as bioleaching period approached to a value of 2.5 to 9, 2.9 to 8.9, 3 to 8.6 and 3.1 to 8.4 in case of glucose, molasses, and saw dust and cotton seed as substrate.

#### **Mass balances**

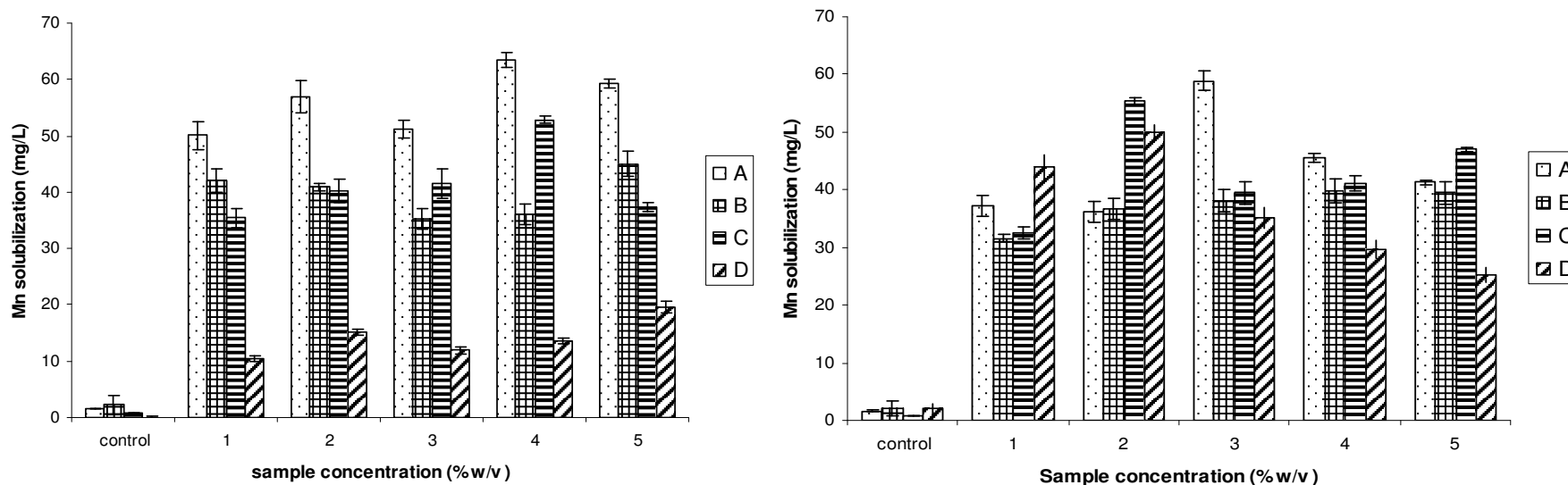
Analysis of residual solids after leaching was carried out to perform a mass balance for Cu, Al, Mn and Mg. The amount of Cu was determined in the residual solids and then added to the maximum amount of the Al, Mn and Mg solubilized. This value was then compared to the initial amount of Cu, Al, Mn and Mg in the raw sample. On the average, some amounts of Cu (28 %), Al (15 %), Mn (19.5%) and Mg (8.5%) have not been recovered. During sample preparation and filtration some of the undissolved salts might be lost.

#### **Conclusions**

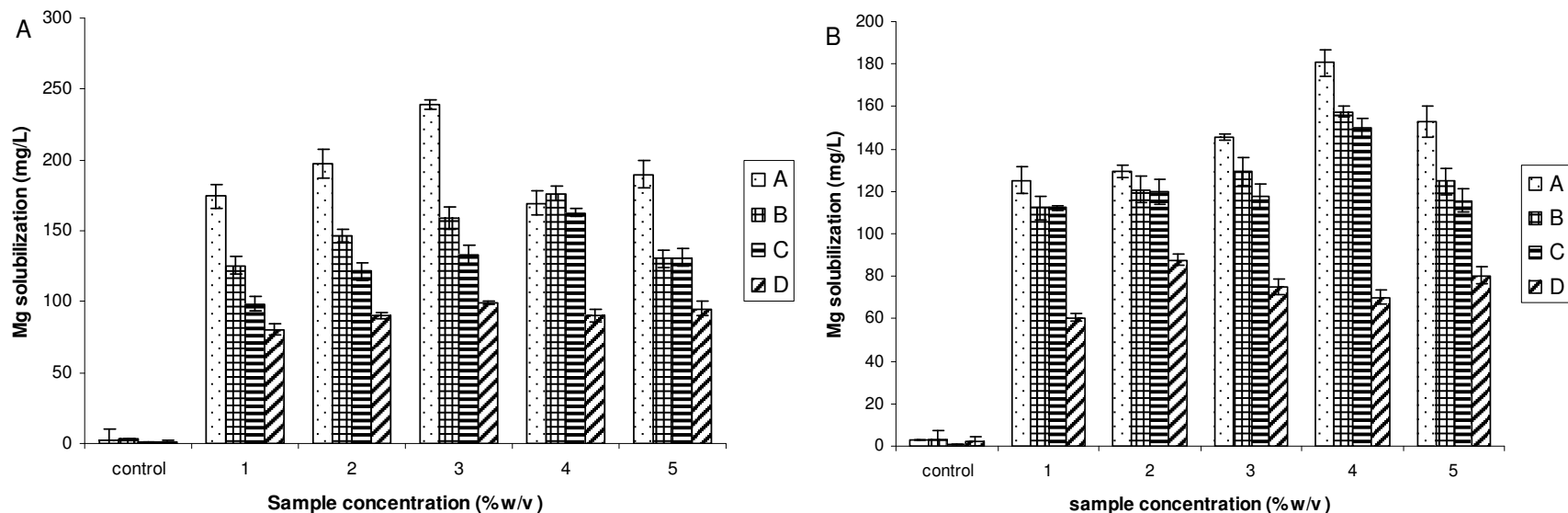
It can be conclude from the above study that recovery of some metal ions from low grade brown shale is feasible. The white rot fungus *G. lucidum* possessed a good potential to secrete some organic acids like tartaric, citric,



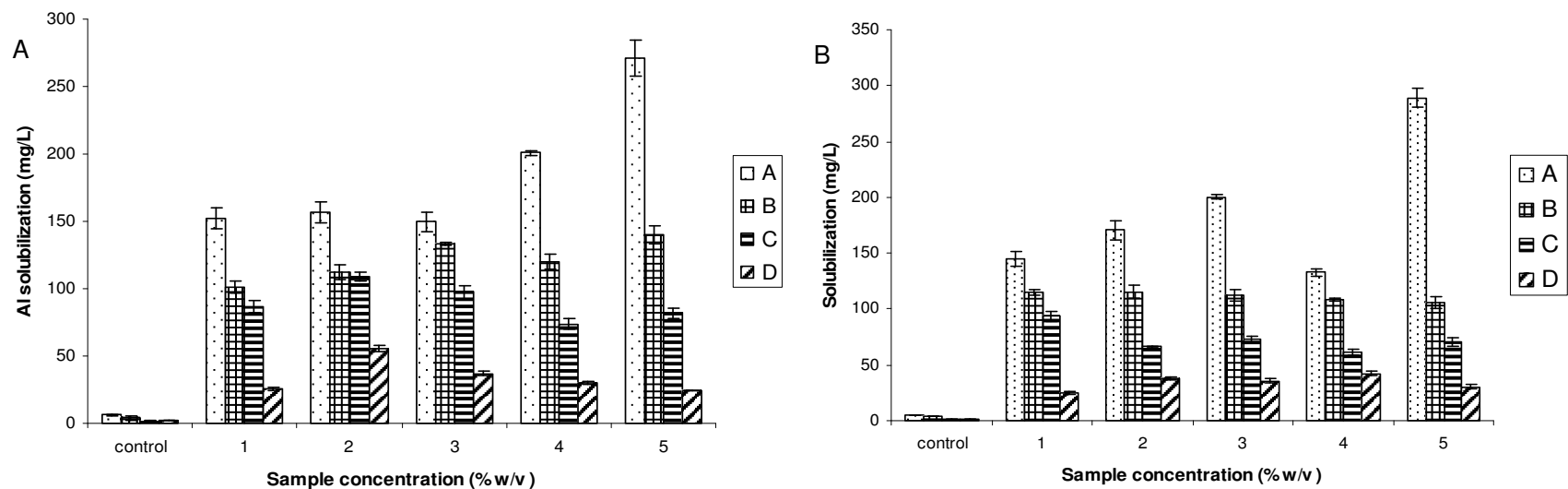
**Figure 2.** (A) Cu solubilization in media A (glucose), B (molasses), C (saw dust) and D (cotton seed cake) at constant substrate concentration in shaking condition; (B) Cu solubilization in media A (glucose), B (molasses), C (saw dust) and D (cotton seed cake) at constant substrate concentration in unshaken condition.



**Figure 3.** (A) Mn solubilization in media A (glucose), B (Molasses), C (saw dust) and D (cotton seed cake) at constant substrate concentration in shaking condition; (B) Mn solubilization in media A (mlucose), B (molasses), C (saw dust) and D (cotton seed cake) at constant substrate concentration in unshaken condition.



**Figure 4.** (A) Mg solubilization in media A (glucose), B (molasses), C (saw dust) and D (cotton seed cake) at constant substrate concentration in shaking condition; (B) Mg solubilization in media A (glucose), B (molasses), C (saw dust) and D (cotton seed cake) at constant substrate concentration in unshaken condition.



**Figure 5.** (A) Al solubilization in media A (glucose), B (molasses), C (saw dust) and D (cotton seed cake) at constant substrate concentration in shaking condition; (B) Al solubilization in media A (glucose), B (molasses), C (saw dust) and D (cotton seed cake) at constant substrate concentration in unshaken condition.

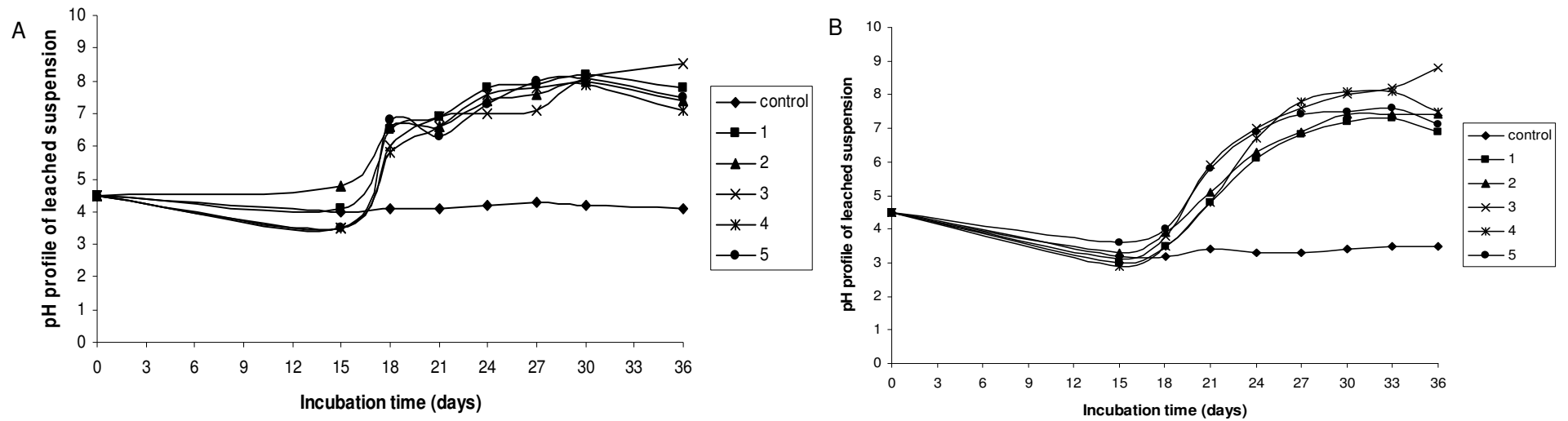


Figure 6. (A) pH profile of glucose medium for shaking culture; (B) pH profile of glucose medium for unshaken culture.

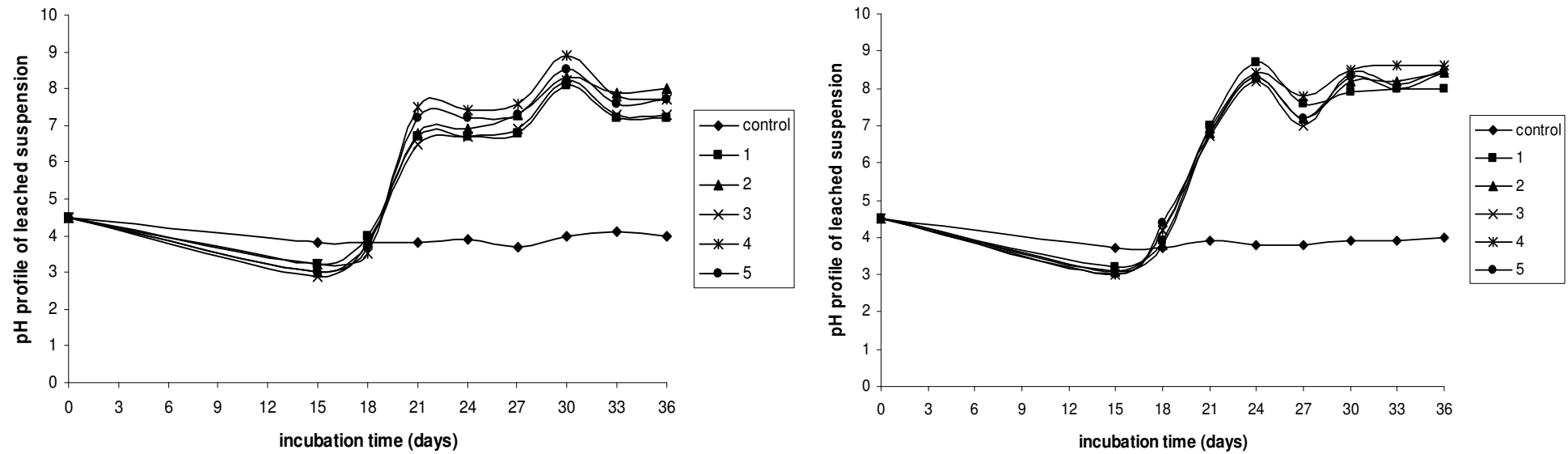


Figure 7. (A) pH profile of molasses medium for shaking culture; (B) pH profile of molasses medium for unshaken culture.



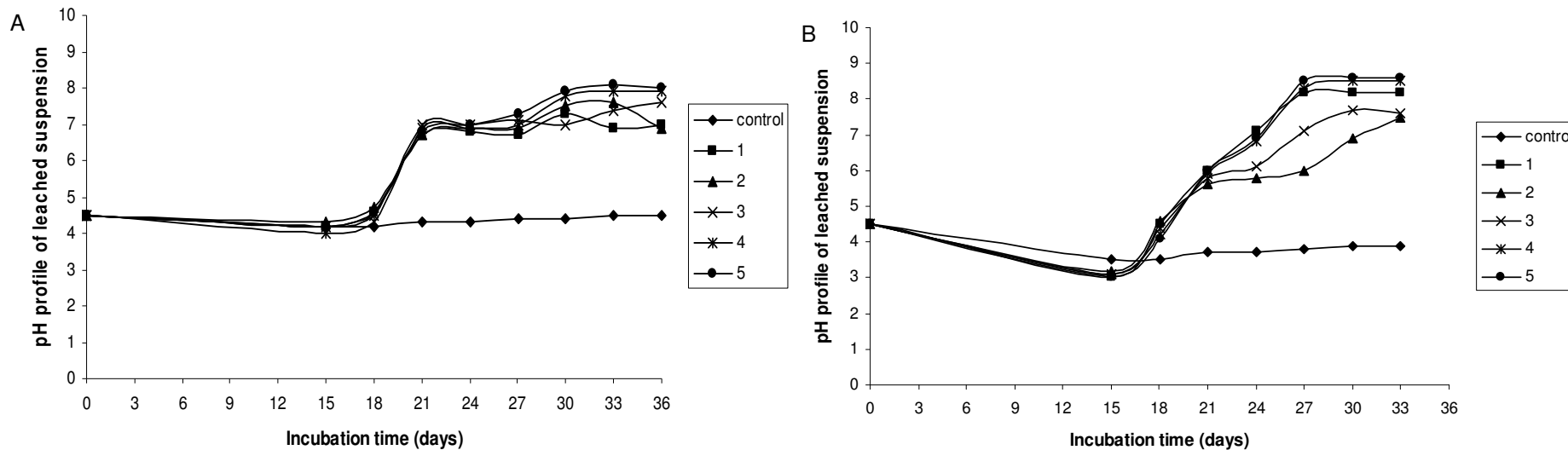


Figure 8. (A) pH profile of saw dust medium for shaking culture; (B) pH profile of saw dust medium for unshaken culture.

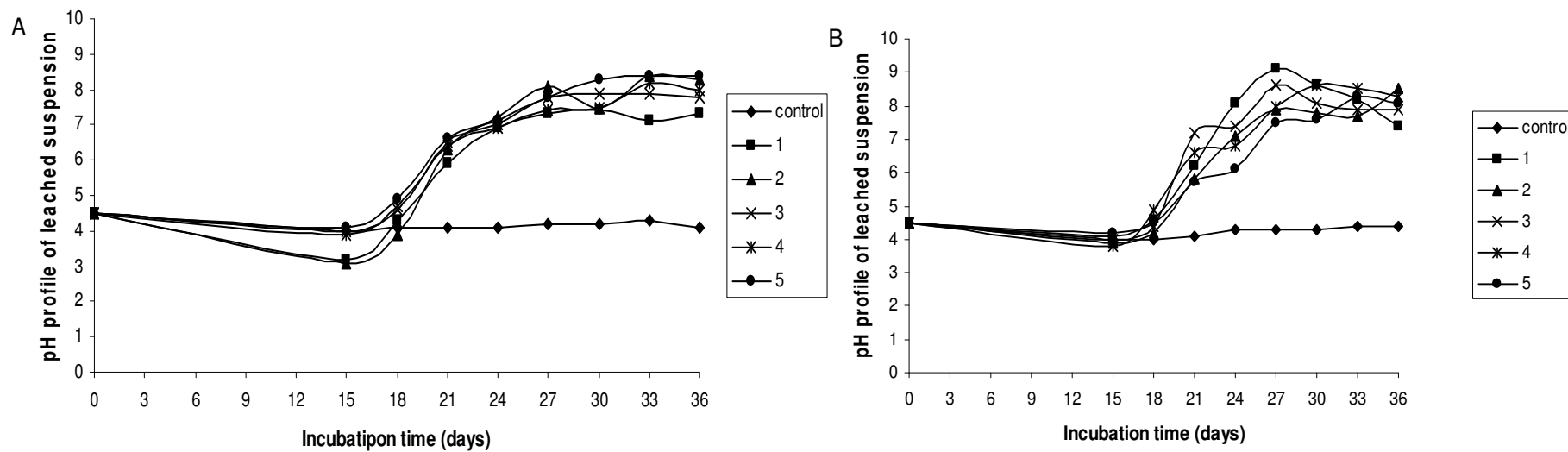


Figure 9. (A) pH profile of cotton seed cake medium for shaking culture; (B) pH profile of cotton seed cake medium for still culture.

oxalic which leached metals ions from the ore. Significant difference in leaching of metals ion by *G. lucidum* was observed under shaking and non shaking conditions. Maximum leaching of Al (90.7%), Mg (96.46%), Mn (66.3%) and Cu (73.45%) was observed using glucose under shaking conditions with 5, 3, 4 and 3% pulp densities respectively.

## ACKNOWLEDGEMENT

The authors are thankful to the Higher Education Commission (HEC) of Pakistan for financial support under the Indigenous 5000 Ph.D. Fellowship Program.

## REFERENCES

- Acharya C, Kar RN, Sukla LB and Misra VN (2004). Fungal bioleaching of manganese ore. *Trans. Indian Inst. Met.*, 57 (5): 501-508.
- Ahmad Z (1969). Directory of mineral deposits of Pakistan. Geological Survey of Pakistan: Records, 3(15): 65-77.
- Anjum F, Bhatti HN, Ghauri MA, Bhatti IA, Asgher M, Asi MR (2009). Bioleaching of copper, cobalt and zinc from black shale by *Penicillium notatum*. *Afr. J. Biotechnol.*, 8(19): 5038-5045.
- Anjum F, Bhatti HN, Ghauri MA (2010). Enhanced bioleaching of metals from black shale using ultrasonics. *Hydrometallurgy*, 100: 122-128.
- Bhatti HN, Anjum F, Saeed S and Rehman K (2009). Bioleaching of rock phosphate by *Aspergillus niger*. *Asian J. Chem.*, 21 (8): 6029-6040.
- Bibi I, Bhatti HN, Asgher M (2009). Decolorization of direct dyes with manganese peroxidase from white rot basidiomycete *G. lucidum*-IBL-5. *Can. J. Chem. Eng.*, 87: 435-440.
- Bohacs KM, Carroll AR, Neal JE, Mankiewicz PJ (2000). Lake basin type source potential and hydrocarbon character: an integrated sequence stratigraphic geochemical framework. In: Gierlowski-Kordesch, EH, Kelts, KR (Eds.), *Lake basin through space and time: AAPG Stud. Geol.*, (46): 3-34.
- Bosshard PP, Bachofen R, Brandl H (1996). Metal leaching of fly ash from municipal water incineration by *Aspergillus niger*. *Environ. Sci. Technol.*, 30: 3066-3070.
- Burgstaller W and Schinner F (1993). Leaching of metals with fungi. *J. Biotechnol.*, 27: 91-116.
- Catherine NM, Kamali M (2003). Bioleaching of copper and other metals from low-grade oxidized mining ores by *Aspergillus niger*. *J. Chem. Technol. Biotech.* 78(5): 497-503.
- Catherine AM, Kamali M, Gibbs FB (2004). Bioleaching of heavy metals from a low grade mining ore using *Aspergillus niger*. *J. Hazard. Mater.*, 110: 77-84.
- Devasia P, Natarajan, KA (2004). Bacterial leaching: biotechnology in mining industry, General article. *Resonance*, 27-34.
- Escobal A, Gonzalez J, Iriondo C, Laborra C (1996). Liquid chromatographic determination of organic acids in txakoli from Bizkaia. *Food Chem.* 4(58): 381-384.
- Galkin S, Vares T, Kalsi M, Hatakka A (1998). Production of organic acids by different white-rot fungi as detected using capillary zone electrophoresis. *Biotechnol. Tech.*, 12 (4): 267-271.
- Johnson DB (2006). Biohydrometallurgy and the environment: intimate and important interplay. *Hydrometallurgy*, 83: 153-166.
- Mulligan CA, Kamali M, Gibbs FB (2004). Bioleaching of heavy metals from a low grade mining ore using *Aspergillus niger*. *J. Hazard. Mater.*, 3: 21-27.
- Ndlovu S (2008). Biohydrometallurgy for sustainable development in the African minerals industry. *Hydrometallurgy*, 91: 20-27.
- Olson GJ, Brierley JA, Brierley CL (2003). Bioleaching review part B: progress in bioleaching: applications of microbial processes by the minerals industries. *Appl. Microbiol. Biotechnol.*, 63 (3): 249-257.
- Pradhan N, Das B, Gahan CS, Kar RN, Sukla LB (2006). Beneficiation of iron ore slime using *Aspergillus niger* and *Bacillus circulans*. *Bioresour. Technol.*, 97: 1876-1879.
- Rawlings DE, Dew D, Plessis CD (2003). Biomineralization of metal containing ores and concentrates: review. *Trends Biotechnol.*, 1 (21): 38-44.s
- Rawlings DE (2004). Microbially assisted dissolution of minerals and its use in the mining industry. *Pure Appl. Chem.*, 76: 847-859.
- Steel RGD, Torrie JH, Dickey D (1987). Principles and procedures of statistics. A biomaterial approach, 3<sup>rd</sup> Ed. McGraw Hill Book Co. Inc., New York, USA.
- Sayer JA, Gadd GM (2001). Binding of cobalt and zinc by organic acids and culture filtrates of *Aspergillus niger* grown in the absence or presence of insoluble cobalt or zinc phosphate. *Mycol. Res.*, 105: 1261-1267.
- Schimizona H (1955). Oxalic acid decarboxylase, a new enzyme from the mycelium of wood rotting fungi. *J. Biochem. (Tokyo)*, 42: 321-340.
- Shull KE, Guthan GR (1967). Rapid modified Eriochrome Cyanine R method for determination of Al in water. *J. Am. Water works Assoc.*, 59: p. 1456.
- Takao S (1965). Organic acid production by Basidiomycetes. Screening of acid producing strains. *Appl. Microbiol.* 13: 732-737.
- Tang JA, Valix M (2006). Leaching of low grade limonite and nontronite ores by fungi metabolic acids. *Miner. Eng.*, 19: 1274-1279.
- Wasay SA, Barrington SF, Tokunaga S (2001). Organic acids for the *in situ* remediation of soils polluted by heavy metals: soil flushing in columns. *Water Air Soil Pollut.*, 127: 301-314.
- Wilscher S, Bosecke K (2003). Studies on the leaching behaviour of heterotrophic microorganisms isolated from an alkaline slag dump. *Hydrometallurgy*, 71: 257-264.
- Wong KK, Lee CK, Low KS, Haron MJ (2003). Removal of Cu and Pb by tartaric acid modified rice husk from aqueous solutions. *Chemosphere*, 50(1): 23-28.
- Xu TJ, Ting YP (2009). Fungal bioleaching of incineration fly ash: metal extraction and modeling growth kinetics. *Enzyme Microb. Technol.*, 44 (5): 323-328.
- Yang J, Wang Q, Wu T (2005). Heavy metals extraction from municipal solid waste incineration fly ash using adapted metal tolerant *Aspergillus niger*. *J. Bioresour. Technol.*, 100 (1): 254-260.