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## A Competitive Effect of Cu (II) and Pb (II) on Biosorption by *Aspergillus Niger* NCIM (616) Using ICPMS

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**ABSTRACT**

Environmental pollution due to heavy metals is a major problem of the century. Lead and Copper due to their extensive industrial usage has become the potential pollutants. Industrial effluents containing toxics and heavy metals drain into the river, which is often the source of drinking water for other downstream .Municipal water treatment facilities in most of the developing countries, at present, are not equipped to remove traces of heavy metals, Release of these pollutants without proper treatment poses a significant threat to both environment and public health, as they are non biodegradable and persistent. Through a process of bio-magnification, they further accumulate in food chains. Thus their treatment becomes inevitable and in this endeavor, biosorption seems to be a promising alternative for treating metal contaminated waters. The purpose of this paper is to present the experimental work on the competitive effect of metals copper and lead by biomass of Aspergillus niger using Inductively coupled plasma mass spectrometry (ICPMS).

**Keywords:** A. niger, Cu (II) , Pb (II), Biosorption and ICPMS.

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## INTRODUCTION

Heavy metals are continuously released into the aquatic environment from natural processes like volcanic activity and weathering rocks . Industrial processes have greatly enhanced the mobilization of heavy metals. Excess heavy metals are introduced into aquatic ecosystems as by-products of industrial processes and acid-mine drainage residues. They are highly toxic as ions or in compound forms: they are soluble in water and may be rapidly absorbed into living organisms [1] after absorption; these metals can bind to vital cellular components, such as structural proteins, enzymes and nucleic acids, and interfere with their functioning. The discharge of non-biodegradable heavy metals into water is a major concern because they tend to accumulate in living organisms, causing various diseases and disorders thereby interfering with the designated best use of water.

Since most of heavy metals are non-degradable into non-toxic end products, their concentrations must be reduced to acceptable levels before discharging them to the environment. Otherwise, these could pose threats to public health and /or affect the aesthetic quality of potable water. Microorganisms have evolved various measures to respond to heavy-metal stress via processes such as transport across the cell membrane, biosorption to cell walls and entrapment in extracellular capsules, precipitation, complexation and oxidation–reduction reactions [2-6]. High metal-binding capacities of several biological materials have already been identified [7], have described the many ways in which bacteria, fungi and algae can take up toxic metal ions.

Biosorption is a physiochemical process that occurs naturally in certain biomass which allows it to passively concentrate and bind contaminants onto its cellular structure because of negative charged groups within its fabric. Though using biomass in environmental cleanup has been in practice for a while, scientists and engineers are hoping this phenomenon will provide an economical alternative for removing toxic heavy metals from industrial wastewater and aid in environmental remediation. Biosorption could be generally defined as the removal of metal or metalloid species, compounds and particulate from solution by biological materials. The term “biosorption” is inadequate since it encompasses, metal adsorption and chemical deposition as well as intracellular uptake by cells Microorganisms including bacteria, algae, fungi and yeast can efficiently accumulate heavy metals and radio nucleotides from their external environment. Filamentous fungi like *A. niger, Rhizopus arrhizus etc.,*are being used in fermentation industries to produce various metabolites , such as lipase, fumaric acid, lactic acid, steroids and celluloses. These biomasses are found to be effective in the removal of heavy metals, radioactive elements. It has been proved that they are capable of adsorbing heavy metals from aqueous solutions, especially for the metal concentration below 50 mg /L .The metal binding capacities of several biological materials have been identified to be very high, including fungi, bacteria, marine algae, yeasts. It was reported that these microorganisms can accumulate a wide range of metals through this process called biosorption.

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Different types of science background, from engineering to biochemistry, can make a significant contribution in elucidating the biosorption phenomenon. Interdisciplinary efforts are mandatory and represent quite a challenge. Preparing biosorption for application as a process requires mainly chemical engineering background. Biosorption phenomenon found to be very useful in removing heavy metals.

In the present study , biosorption of Pb (II) & Cu (II) is carried out using *A.*NCIM 616 under SMF and also studied the effect of paramaters such as pH, temp , time, substrate concentration using(Inductively coupled plasma mass spectrometry) ICPMS which were influenced more at the removal of Pb (II) & Cu (II) and the comparision between these metals.

## MATERIALS AND METHODS

The experimentation is carried out on batch-wise by Submerged fermentation , on biosorption of lead and copper using ICPMS by an biosorbent *A. niger* NCIM 616 ,

**Microorganism** *A.niger* NCIM 616, procured from National Collection of Industrial Microorganisms (NCIM), NCL, Pune.

**Maintainance Medium**

**Potato Dextrose Medium**

| Composition | Grams/l |
|---|---|
| Potato | 200g |
| Dextrose | 20g |
| Yeast Extract | 0.1g |
| Agar | 20g |
| pH | 5.6 |

The organism was sub-cultured at regular intervals of 4 weeks. The culture was grown at 28$^{o}$ C for 5 days and stored at 4$^{o}$ C for further use.

**Maintenance Conditions:** Growth Conditions: Aerobic & Anaerobic, Temperature: 28$^{o}$ C, Incubation Time: 96 hrs, Sub-culture: 4 weeks

**Maintenance of Culture:** The experiments decribed in this study were carried out using *A. niger* NCIM 616 which was maintained on potato dextrose agar (PDA) at 4$^{o}$ C and subculture was done frequently in the laboratory . Fresh slants were prepared for running experiment.

**Cultivation of fungi species for biomass production:** The basal medium contained (g/dm$^3$): ammonium nitrate, 2.06; monopotassium phosphate, 0.55; $MgSO_4.7H_2O$, 0.25; sucrose, 50. The basal medium was made up with distilled water. The medium was swirled while still hot and then allowed to stand overnight. This medium was then adjusted to pH 5 using either NaOH or

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HCL and distributed into 250 ml conical flasks. The media in all the flasks were autoclaved at 121$^{o}$ C , 15lbs, for 20 min.

For the transfer of fungal cultures, spore inoculum was prepared by taking a loopsfull of spores from the slants of 5 day mother culture using inoculating loop in sterilized conditions. Tween80 acts as surfactant. In each conical flask, 10 ml of spore inoculums was added. Then these flasks were incubated in orbital shaker (100 rpm) at 28$^{o}$ C for 4 days.

**Preparation of the microorganism for biosorption**

After 4–5 days of growth, the harvested cells were washed with generous amounts of deionized distilled water till the pH of the wash solution was in the near neutral range . Then, it was dried at 60 ◦C for 24 h before use. Ten grams of dried microorganism was suspended in 100 dm−3 deionized distilledwater and homogenized for 20 min in a homogenizer at 8000 rpm for 20 min and then stored in the refrigerator at 4 ◦C.

**Incubation of fungal biomass with metal ion**

A microorganism suspension of 10 cm$^3$ was mixed with 90 cm3 of solution containing aknown concentration of metal ions in 250 cm$^3$. Erlenmeyer flasks at desired temperature and pH for evaluating their influence on metal adsorption. All the final solutions contained a fixed concentration of biosorbent (1.0 g dm−3). the pH was adjusted to. The flasks were agitated on a shaker at a 100 rpm constant shaking rate for 7 days.

**Metal determination**

Samples of 5 cm3 were taken at predetermined time intervals for the residual metal ion concentrations in the solution. Before analysis the samples were centrifuged at 4000 rpm for 3 min and the supernatant fraction was analyzed for the remaining metal ions. All experiments were carried out at least twice. Values used in the calculations were the arithmetic averages of the experimental data. The residual Cu(II) and Pb(II) ions in the biosorption media were determined by using an atomic adsorption spectrophotometer (UNICAM 929) and Inductively coupled plasma mass spectrometry (AGILENT). .Thus the samples are collected and digested using Conc. $HNO_3$ for metal concentrations in ICPMS. The amount of metal taken up by the biomass was calculated as the difference between the initial and final concentration of the metal in the aqueous solution.

**Inductively coupled plasma mass spectrometry**

Inductively coupled plasma mass spectrometry (ICP-MS) is a type of mass spectrometry which is capable of detecting metals and several non-metals at concentrations as low as one part in $10^{12}$ (part per trillion). This is achieved by ionizing the sample with inductively coupled plasma and then using a mass spectrometer to separate and quantify those ions.

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## RESULTS AND DISCUSSION

### Optimization of fermentation process

Experiments were carried out by *A. niger* with heavy metals such as Lead nitrate and Copper Sulphate for maximizing the reduction of metal concentration by optimizing the process parameters under submerged state fermentation. Optimization was done on "one parameter a time basis" i.e., by changing one independent variable while fixing the others at a certain constant level. The optimum conditions obtained in each parameter was applied to the subsequent experiments. All the experiments were conducted in triplicate and the mean values are reported.

### Effect of pH on biosorption:

The most important parameter influencing the sorption capacity is the pH of adsorption medium . The initial pH of adsorption medium is related to the adsorption mechanisms onto the adsorbent surface and reflects the nature of the physicochemical interaction of the metal in solution and the adsorptive sites of adsorbent. The impact of the solution pH on the metal biosorption was investigated in the biomass *A. niger* NCIM 616 . Since pH is one of the main variables affecting the biosorption process [8], the optimum pH value for the uptake of metals was determined. Six different pH tests, chosen within the solubility range of the metals used, were carried out. Growth conditions of this species was optimized at various pH(3-7).The pH of the culture broth dropped significantly as compared to the control, where it remained constant at 7. At pH higher than 4.0, lead ions precipated , and thus maximum adsorption of 12.37 mg/g (ICPMS) was seen only at pH 4 where as Copper ions maxiumum adsorption of 9.18 mg/g (ICPMS) was seen at pH 5.

Adsorption of lead & copper ions respectively over the pH range 4–5, pH-related effects were significant (Fig. 3.1). Meanwhile, at the pH values of 3, the adsorption values started to decrease. At low pH, protons would compete for active binding sites with metal ions. The protonation of active sites thus tends to decrease the metal sorption. At a low pH, of almost 2 & 3, all the binding sites may be protonated, thereby desorbing all originally bound metals from the biomass [9, 10]. An additional possible explanation why sorption increases with increasing pH is that the solubility of many metals in solution decreases with increasing pH. A further possible explanation of increasing sorption with increasing pH is that hydrolyzed species have a lower degree of hydration, i.e. less energy is necessary for removal or reorientation of the hydrated water molecules upon binding [11].At a further increase of pH (6–7) the solubility of metals decreases enough for precipitation to occur (Table 3.1). This should be avoided during sorption experiments as distinguishing between sorption and precipitation metal removal becomes difficult [11].

Kapoor and Viraraghavan [12] reported that amine and carboxyl groups are important functional groups involved in biosorption of heavy metals by *A. niger* and biosorption of heavy

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metals was severely inhibited when these groups were modified. At highly acidic pH, the overall surface charge on the cells became positive and metal cations and protons compete for binding sites on cell wall, which results in lower uptake of metal. It has been suggested that at low pH values, cell wall ligands would be closely associated with H3O+ that restrict access to ligands by metal ions as a result of repulsive forces. At pH values above the isoelectric point, there is a net negative charge on the cell surface and the ionic state of ligands such as carboxyl, phosphate and amino groups will be such that so as to promote reaction with metal ions, hence the rapid binding efficiency was obtained.

At various pH , the following adsorbance of lead and copper was observed under ICPMS in Fig.1

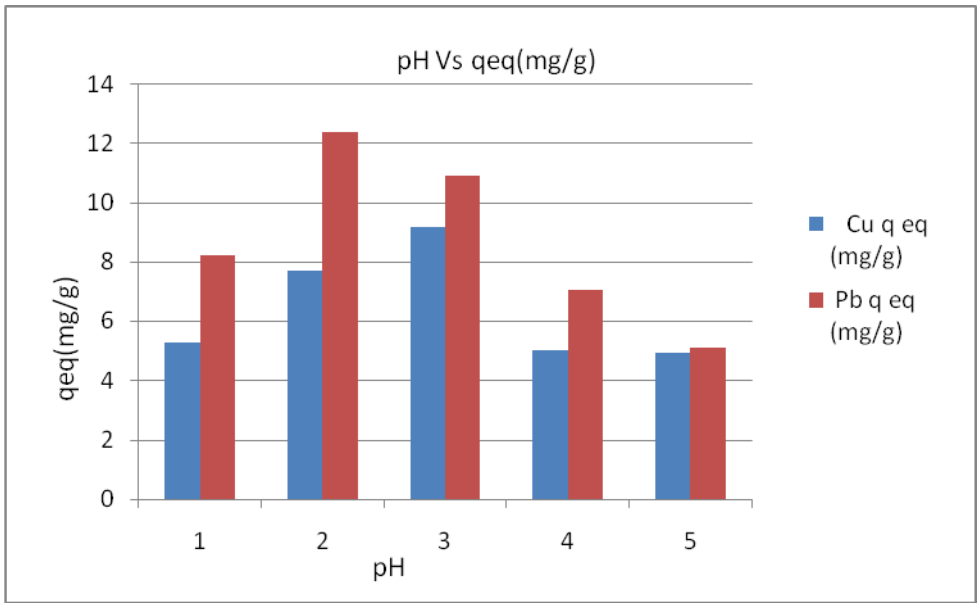


**Fig 1. pH Vs qeq studied in ICPMS**

**Effect of Temperature on Biosorption**

Removal of metal ions- Pb(II) & Cu(II) by the biomass were carried out experimentally at different temperatures ranging from 20 to 35 $^{o}$C are shown in fig. At low temperatures , the binding of Pb(II) & Cu(II) ions to *A.niger* NCIM 616 was by passive uptake. Maximum initial adsorption rate of Pb(II) & Cu(II) ions was obtained at temperature 35$^{o}$C. A decrease in reduction of metal concentration was observed when the incubation temperature was higher or lower than the observed optimum incubation temperature. At 35 $^{0}$C , maximum amount of 9.18 mg/g(ICPMS) of Cu and 12.36 mg/g(ICPMS) of Pb(II) was adsorbed by dried microorganism. These adsorption data were further fitted to two adsorption models to find out the suitable model.

Gupta et al.,[13] maximum amount of adsorption was reported at 35$^{0}$C .

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It was shown in Fig.2 that the removal of both metal ions increased with increasing temperature up to $35^0$C.

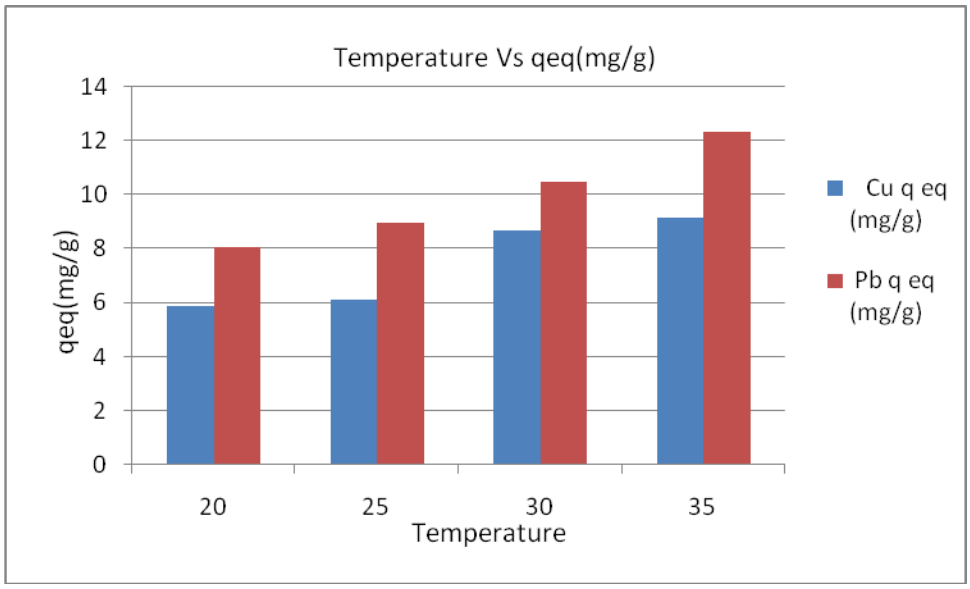


**Fig 2. Temp Vs qeq studied in ICPMS**

**Effect of time**

To determine the optimal biosorption time, samples were periodically taken at one day interval . The results are presented in Fig 3.3. The results indicate that from 1 hr to 3 hr , there was very less reduction in the metal concentration . The rate of reduction of metal concentration increased by 2 fold on 4 $^{th}$ hr , increased gradually and attained high reduction (mg/g) after 6 hrs and continued upto 8 hrs of incubation with *A. niger* . Thereafter, further increase in time increased the metal concentration.

The decrease in reduction of metal concentration after an optimum incubation was probably owing to a reduced growth rate from fast depletion of nutrients available to the organism, and also could be owing to the production of secondary metabolites resulting in lower enzyme activity.

Fig 3 shows the biosorption kinetics of Pb(II) and Cu(II) ion removal at various intervals of time at previously optimized conditions i.e., at pH 4 & 5 at 35 $^{o}$C by plotting the metal ion uptake capacity (*q*) versus time. The biosorption capacity increased with increasing contact time and a larger amount of metal ions were adsorbed by 8 hrs of contact time. Equilibrium was established after 8 hrs of contact time. After an equilibrium was established, no more Cu(II) & Pb(II) were adsorbed.

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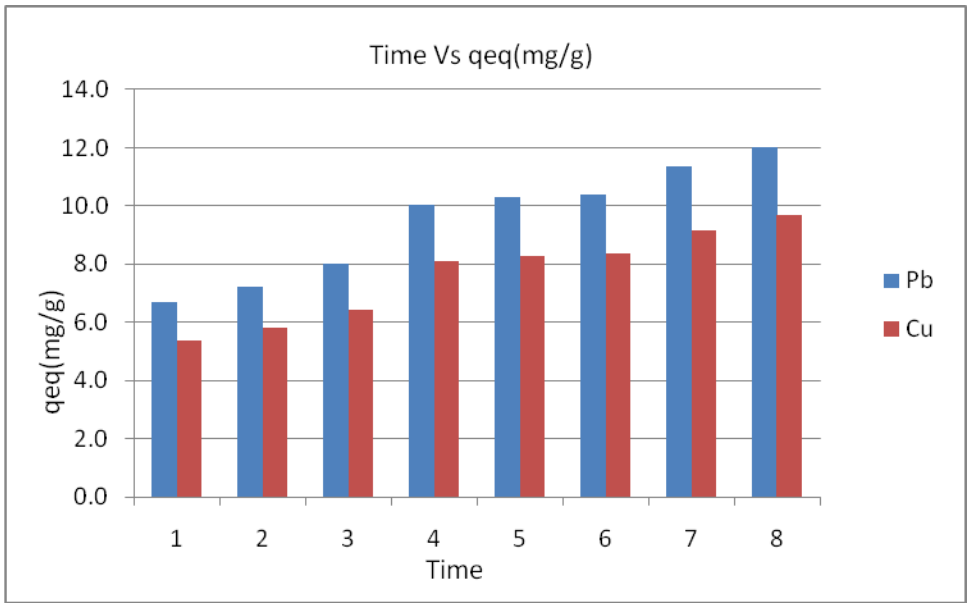


**Fig 3. Time Vs qeq studied in ICPMS**

## CONCLUSION

The ability of *A. niger* NCIM 616 to adsorb Pb(II**)** & Cu(II**)** was investigated in a batch system. It was seen that pH, temperature and time highly affected the biosorption capacity of the sorbent. The studies indicate that *A.niger* NCIM 616 is an effective biosorbent for Pb(II) & Cu(II) removal. The maximum Pb (II) biosorption capacity has been found to be 60.7% Pb(II) of dry weight of biomass at an fungal dose of 20 mg/ $dm^3$ in of contact time 8 days and optimum pH of 4.0 at temp $35^0$ C.. The maximum Cu(II) biosorption capacity has been found to be 48.37% Cu(II) of dry weight of biomass at an fungal dose of 20 mg/$dm^3$ in of contact time 8 days and optimum pH of 5.0 at temp $35^0$ C. This shows that *A. niger* NCIM 616 has greater adsorbing capacity for Pb(II) than Cu(II) . Accurate results of biosorption of metals were obtained in ICPMS (AGILENT)

## REFERENCES

[1] Agarwal A and Sahu KK. J Hazard Mat 137, 915-924(2006) .
[2] Rai LC, Gaur JP, Kumar HD. J Biol Rev 1981;56:99 – 151.
[3] Huang C and Huang CP. J Water Res 1996;30(9):1985-1990.
[4] Avery SV, Tobin JM. J Appl Environ Microbiol 1993;59:2851– 6.
[5] Brady D, Duncan JR. J Biotechnol Bioeng 1994;44:297– 302
[6] Krauter P, Martinelli R, Williams K, Martins S. J Biodegradation 1996;7:277– 86.
[7] Gadd GM (1990). H. Ehrlich & C. L. Brierley. Fungi and yeasts for metal accumulation. J. of Microbial Mineral Recovery , McGraw-Hill, New York, pp. 249-275.
[8] Lezcano JM, Gonzalez F, Perez I, Blazquez ML, Munoz JA, Ballester A, Hammaini A. Biohydrometallurgy: fundamentals, technology and sustainable development, Part B 2001: 217–226.

*ISSN: 0975-8585*

[9] Gupta R, Ahuja P, Khan S, Saxena RK, Mohapatra H. Curr Sci 2000 78, 967–973
[10] Aldor I, Fourest E, Volesky B. J Chem Eng 1995; 73: 516–522.
[11] Schiewer S, Volesky B. J Environ Sci Technol 1995; 29: 3049–3058.
[12] Kapoor A & Viraraghavan T. J Bioresour Technol 1995; 53:195-206.
[13] Gupta R, Ahuja P, Khan S, Saxena RK, Mohapatra H. Curr Sci 2000; 78:967–973