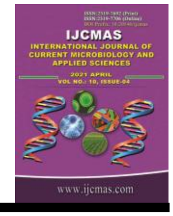
Int.J.Curr.Microbiol.App.Sci (2021) *10*(04): 890-902

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 10 Number 04 (2021)**
Journal homepage: http://www.ijcmas.com



Original Research Article https://doi.org/10.20546/ijcmas.2021.1004.096

Removal of Heavy Metals using the Isolates of *Aspergillus* sp. Isolated from Contaminated Pulp and Paper Mill Sludge

M. Ezhilvanan[1]*, S. F. Lesley Sounderraj[1] and Nancy Lesley[2]

[1]*Department of Zoology, Voorhees College, Vellore, Tamil Nadu, India*
[2]*Department of Statistics, Presidency College, Chennai, Tamil Nadu, India*

**Corresponding author*

Keywords

Bioaccumulation, Biosorption, Pulp and paper mill sludge (PMS), Pulp and paper mill effluent (PME)

Article Info

Accepted:
12 March 2021
Available Online:
10 April 2021

A B S T R A C T

Industrial discharges, in the form of effluent is one of the greater problems causing serious environmental pollution. Pulp and paper mills are categorized as one of the 12 most polluting industries containing heavy metals like Cu, Zn, Cd, Pb, Cr and Mn. The removal and recovery of heavy metals from effluent is indispensable for the protection of environment. Biological methods such as bioaccumulation and biosorption of heavy metals provide an alternative to physical and chemical methods for waste water treatment. In the present study, *A.flavus* and *A.fumigatus* isolated from pulp and paper mill sludge showed tolerance and accumulation of toxic metals from synthetic medium and paper mill effluent. Effect of heavy metal ions on fungal growth in terms of their biomass (dry weight) was determined and conformed energetic fungal growth after increasing the concentration of Pb^{2+} and Zn^{2+} point out the importance of these two fungi for bioremediation. Heavy metal reductions were found significant ($P<0.001$) in paper mill effluent treated with *A.flavus* and *A.fumigatus,* and accumulated maximum Pb followed by Cu, Zn, Cd, Cr and Mn metals were noticed.

Introduction

A serious problem of environmental pollution has arisen in recent years, due to heavy metals resulting from many industrial effluents such as smelting, mining, metal plating, pigment and metallurgical (Akar and Tunali, 2006).The effluents released from the paper mills contain environmentally hazardous heavy metals and other organic toxicants (Verma *et al.*, 2005). The concentration of Cu, Cr (VI) and Pb was significantly high in paper mill effluent due to its application as catalyst, pigments, wood preservatives and corrosion inhibitors (Goel, 1996).According to Leung *et al.*, (2000) and Lacina *et al.*, (2003), the fungi especially *Aspergillus species* have been proved to be more efficient and economical in removal of metals and organic toxicants from (dilute) aqueous solutions compared to conventional methods, because of their filamentous and spherical morphological mycelia and high cell wall percentage with functional groups like amine, carboxyl and phosphate which influence the heavy metal adsorption. *Aspergillus sp*. accumulate micronutrients such as Cu, Zn, Mn and toxic metals like Ni, Cd, Sn and Hg in amounts higher than the nutritional requirement. In light of the above, the present study was carried out to investigate the metal accumulation and biosorption potential of *A.flavus* and *A.fumigatus* isolated from pulp and paper mill sludge.

Materials and Methods

Sample collection

The untreated paper mill effluent (PME) was collected from the final discharge point (Plate: 1, 2 &3) of the effluents of Seshasayee Paper, Board and Pulp mill Ltd, Pallipalayam, situated at 11^{o}20’27’’North 77^{o}43’02’’East and north-west of Erode, Tamil Nadu, India, which produces board, printing and writing paper as its main products from bagasse based integrated mill, having an installed capacity of 1,20,000 t/annum. The samples were collected(Sludge and effluent) in dry sterilized Petridish and 20 litres polypropylene container respectively. Samples were preserved at 4^{o}C in the refrigerator to retard biological activity prior to use till its processing for isolation of heavy metals resistant fungi.

Physico-chemical analysis of paper mill effluent

The sample of untreated paper mill effluents were analysed using the standard methods prescribed by APHA (2005) for different Physico-chemical parameters, viz. colour, pH, turbidity, total dissolved solids(TDS), calcium, Magnesium, Reactive Silica SiO_2, chemical oxygen demand (COD), Chlorides, sulphate, potassium, sodium, manganese, zinc, chromium, copper, cadmium and Lead.

Determination of heavy metals

Metal treated filtrate medium was digested using 5ml of concentrated HNO_3 and boiling chips. The content was boiled and evaporated to 16-20ml on hot plate. 5ml of concentrated HCl was added and boiled till sample become clear and brownish fume was evident. Then dried container was cooled, diluted with 100ml double distilled water and filtered through Whatman’s No.1 filter paper.

Concentration of heavy metals in the filtered solution was determined using AAS instrument (Srivastava and Thakur, 2006). The dried fungal matt was crushed in pestle and mortar. Ground material was placed in conical flask and 5:5 ratio of nitric/perchloric acid mixture was added. The content of the flask was placed on hot plate until the production of red nitrous fumes ceased and liquid becomes colourless. Finally the container was cooled, diluted to 100ml with double distilled water and filtered through Whatman’s No.1 filter paper to analyse heavy metals content using AAS instrument (Juwarkar, 1988).

Isolation and Identification of heavy metal tolerance strains

10g of sludge sample was serially diluted and made up to 10^{-1} to 10^{-6} using sterile distilled water. A sample of 0.1ml from the appropriate dilution was spread on CzapeckDox’s agar plates and the plates were incubated at room temperature for 5-7 days. After incubation, the distinct colonies were sub cultured continuously on the suitable medium for the isolation of pure culture. The pure culture strains of isolated fungi were tested for heavy metal tolerance by placed these stains in CzapeckDox’s agar medium supplemented with individual heavy metals in the form of salts $ZnSO_4$, $CdCl_2$, Pb NH_3, $K_2Cr_2O_7$ and $CuSO_4$ $5H_2O$ separately at the concentration of 50 ppm. The plates were incubated for 5-7 days at 28°C for their growth. Isolated pure colonies were maintained on CzapeckDox's agar slants and sub-culturing was carried once a month by growing them at 28° C for 7 days and stored in the refrigerator for further use. The spore suspensions were stored as stock culture in 20% W/V glycerol at -20°C.

Screening of potential strains for metal removal

Different fungal isolates from paper mill sludge were grown on the CzapeckDox's agar media(Plate. 7 and 8). Screening of fungal isolates capable for heavy metal removal was carried out using the loopful of fungal growth, from grown culture were embedded on CZA plate containing stress amount of PME and incubated at 28°C ±1°C for 5 days.

Heavy metal degrading ability of each fungal isolate was identified by the presence of clear zone around their colonies. These fungal stains were identified based on their morphology and reproductive structural characteristics (Nagamani *et al.,* 2006).

Preparation of metal solutions

Stock metal solutions of 1000mg l^{-1}concentration of Zn, Cd, Pb, Cu and Cr were prepared by dissolving analytical grade of salts of $ZnSO_4$ $6H_2O$, $CdCl_2$, $(CH_3COO)_2$, Pb_3H_2O, $CuSO_45H_2O$ and $K_2Cr_2O_7$ separately in 1 litre of double distilled water. The desired (100, 250, 500 and 1000 mgl^{-1}) concentrations of working metal solution were prepared from stock solution. Before mixing with media and fungal culture, pH of each test metal solution was adjusted to desirable value with that of media using 0.1N HCl and 0.1N NaOH.

Bioaccumulation of metals from synthetic medium and paper mill effluent

The spores from a fully sporulated slants of *Aspergillus flavus* and *A.fumigatus* were dispersed separately in 10 ml of sterile water containing 0.1% Tween 80 and by rubbing the spore with a sterile loop under aseptic conditions. The spore suspension showed 1×10^6 spores/ml were inoculated in to the medium in 250 ml Erlenmeyer's flask containing 100 ml of specific production medium (Potato Dextrose Agar for *A.flaveus* and CzapeckDox's for *A.fumigatus*) supplemented with 100,250,500 and 1000 mg l^{-1} concentrated of each heavy metal. Inoculated flasks were incubated on reciprocating shaker at 200rpm at 28°C for 7 days with control flask containing spore inoculated medium without metal salts. Whereas from accumulation of metal from PME, 100mg of biomass of *Aspergillus flavus* and *Aspergillus fumigatus* were inoculated separately into 100 ml of untreated pulp and paper mill effluent enriched with 0.1% of glucose and tryptone for carbon and nitrogen substrates in 250 ml of Frlenmeyers flask. Inoculated samples were incubated with control containing 100 ml of treated effluent without fungal biomass. All the flasks were incubated at 28 °C for 72 hours to check fungal growth and its metal uptaking potential. After incubation concentrations of heavy metals in fungal treated effluent and control was determined to find out any significant heavy metals reduction by fungi compared with untreated paper mill effluent.

Determination of dry weight of fungal biomass

After incubation period, the fungal matt was harvested from working culture by sieving through whatman's filter paper and filtrate medium was collected. Fungal matt was thoroughly washed twice with distilled water to remove non biomass ash and dried in an oven at 80^oC for 12 hours and constant dry weight was taken.

Optimization of parameters

Batch forms of experiments were conducted in Erlenmeyer flasks to determine the effects of p^H, temperature and contact time, metal ions and biomass concentration on bioaccumulation of heavy metals for optimization of parameters in heavy metals bioaccumulation study, the 7 day old fungal spores of *Aspergillus flavus* and *Aspergillus fumigatus* was inoculated into 100 ml of selective medium (PDA and CZA respectively) containing 100 mg l^{-1} concentration of each heavy metal in 250 ml Erlenmeyer flask. After optimization, the conditions of pH, temperature and incubation period was followed for further experiments (removal of heavy metals from untreated PME effluent) and the heavy metals content in filterate medium was determined using AAS (Srivastava and Thakur, 2006).

Results and Discussion

Physico-chemical characteristics of untreated pulp and paper mill effluent

The physical-chemical characteristics of untreated pulp and paper mill effluent are given in the Table 1. The heavy metal characteristics are given in the Table 2.

Heavy Metal tolerance of Fungal Isolates

Effect of Zn, Cd, Pb, Cu and Cr ions on fungal growth in terms of the dry weight of the their biomass was investigated at increasing concentrations of 100 to 1000 mg l^{-1}(Fig.1a,b). *A.flavus* showed heavy metal tolerance upto 1000 mg $l^{-1}$of Zn and Pb followed by 250 mg l^{-1} Cd, 100 mg l^{-1} of Cu and Cr. *A.fumigatus* tolerated 1000 mg l^{-1} of Zn and Pb followed by 250 mg $l^{-1}$of Cd, Cu and Cr. There was an increase in fungal growth in the media amended with Pb and Zn compared with control. The biomass of *A.flavus* and *A.fumigatus* was observed to be high in 100 mg l^{-1} concentration of Pb followed by Zn>Cr>Cu>Cd. The biomass of *A.flavus* and *A.fumigatus* decreased with increasing metal concentration. The vigorous fungal growth towards increasing concentration of Pb and Zn point out the importance of these metals in fungal growth and to exploit these fungi in bioremediation of heavy metals contaminated effluent.

The result showed that increasing the concentration of heavy metals had influence on the fungal biomass and heavy metals accumulation. Both *A.flavus* and *A.fumigatus* showed resistance towards Pb and Zn at high concentration but could not accumulate these metals at higher concentrations. Similarly, Zetic *et al.*, (2001) reported that the effect of heavy metals on fungal growth was variable and depends on the type of metal and its concentration in the medium. The toxicity effect of some heavy metals like Cd, Cu and Cr on fungal growth is due to their strong binding affinity with the cell membrane components which in turn damage the cell integrity and impairment of cell function (Chen and Wang, 2007). This might be the reason for the decreased level of fungal growth which are treated with Cd, Cu and Cr.

Heavy metal accumulation

In the present study, the isolated fungal species of *A.flavus* and *A.fumigatus* from paper mill sludge showed tolerance and accumulation of toxic heavy metals such as Zn, Cd, Pb, Cu and Cr from synthetic medium and paper mill effluent. The metal tolerance and loading capacity of living cells of *A.flavus* and *A.fumigatus* is shown in (Fig. 2a, b). *A.flavus* accumulated high amount of Pb

(88.31%) followed by Zn (48.66%) > Cr (46%) > Cu (36%) and Cd (32.17%) from the concentration of 100 mg l^{-1} of metal solution. Whereas metal uptake from increased concentrations of metal solution such as 250, 500 and 1000 mg l^{-1} was found as follows: Pb (70.6%),> Zn (40.22%) > Cd (17.60%),Pb (56.77%) > (Zn 25.3%) and Pb (32.4%) > Zn (20.27%) respectively. The order of heavy metal accumulation by *A.fumigatus* showed high efficiency towards Pb (89%) followed by Zn (48%) > Cu (42.33) > Cr (39.6%) > Cd (37.75%) Whereas metal accumulation from 250 and 500 mg l^{-1} of metal solution was found in the following order: Pb (63.3%) > Zn (40.46%) > Cu (30.12%) > Cr (23.35%) > Cd (22.45%) and Pb (61.56%), Zn (26.27%) respectively. However, *A.fumigatus* accumulated Pb (41.10%) and Zn (24.18%) from the concentration of 1000 mg l^{-1}.Thippeswamy *et al.,* (2012) reported the accumulation of Pb, Zn, Cu and Ni by *A.Flavus* from synthetic medium. In the present study, *A.fumigatus* showed comparatively higher resistance, growth and uptake of Pb and other metal ions compared with *A.flavus*. In both the organisms, the accumulation of Pb was found to be high as compared to other metal ions.

However in the presence of Cr and Cd, the growth of *A.flavus* was inhibited. There was an increase in fungal growth in the media supplemented with Pb and Zn compared to control in *A.flavus* and *A.fumigatus.* In earlier findings Akar and Tunail (2006) reported only 22% Pb and 20% Cu biosorption by *A.flavus*. Sugasini *et al.,* (2014) investigated the biosorption potential of *Aspergillus* sp. isolated from tannery effluent. In the present study, both *A.flavus* and *A.fumigatus* showed high adsorption capacity of chromium from PME, it may due to the development of adaptation of this fungi to different heavy metal concentrations of PME compared to single metal effluent.

Optimization conditions

Effect of pH

The effect of initial pH on the absorption of Zn, Cd, Pb, Cu and Cr at 100 mg/l on *A.flavus* and *A.fumigatus* was investigated at 26±02^{o} C. As can be seen from (Fig:3a,b) biosorption of Zn and Cd ions increased with solution pH up to 6.0 and biosorption of Pb, Cu and Cr ions increased with solution pHupto 5.0 in *A.flavus*. There were wide variations in the initial pH during the biosorption process. The maximum biosorption capacity by biosorbent on different metals were noted as 45%, 34% of Zn and 58%, 38% of Cd by *A.flavus* and *A.fumigatus* with pH of the solution at 6.0, Whereas the optimal pH for biosorption of heavy metals like Pb, Cu and Cr were noticed as 72%, 46% and 44% respectively with the pH of the solution upto 5.0 in *A.flavus*.

Whereas biosorption of heavy metals like Zn 58% and Cd 38% in *A.fumigatus* with the pH of the solution up to 6.0. The pH dependency of metals up take by *A.fumigatus* shows little higher percentage as Zn 58%, Cd 38%, Pb 76%, Cu 46% and Cr 44% at pH 5-6.0 like as that of *A.flavus*. The pH of the biosorption medium affects the solubility of metal ions and the ionization state of the functional groups (ie. Amine, carboxylate and phosphate groups) of the fungal cell wall (Arica *et al.,* 2003). Because of high concentration of protein at lower pH, heavy metal biosorption decreases due to the positive charge density on metal binding sites, ie. Hydrogen ions complete effectively with metal ions in binding to the sites. The negative charge density on the cell surface increases with increasing pH due to deprotonating of the metal binding sites. The metal ions their complete more effectively for available binding sites, which increases biosorption (Kapoor and Viraraghavan, 1997; Kapoor *et al.,* 1999).

Table.1 Physical-chemical characteristics of untreated pulp paper mill effluent

Parameters	Properties
Colour	Black
pH Value @ 25^{o}C	6.21
Turbidity	41.4
Total Dissolved Solids	3126
Calcium as Ca	533
Magnesium as Mg	20.6
Reactive Silica SiO2	540
Chemical oxygen demand	531
Bio – Chemical Oxygen demand (3 days @ 27^{o} C)	387
Chlorides as Cl^{-}	612
Sulphates as SO_4^{2-}	531
Potassium as K	387
Sodium	531

Table.2 Heavy metal characteristics of untreated mixed Paper mill effluent

Parameters	Mixed paper mill effluent
Manganese	1.815
Zinc	2.036
Chromium	1.476
Copper	1.067
Cadmium	1.040
Lead	2.320

Table.3 Removal of heavy metals (mg l^{-1}) from paper mill effluent treated by *Aspergillus flavus* and *Aspergillus fumigatus*

Heavy metals	*A.flavus* treated effluent	Average % Removal	*A.fumigatus* treated effluent	Average % Removal
Mn	1.105±0.019***	60.86	1.296±0.099**	71.40
Zn	1.329±0.025***	64.95	1.620±0.062***	79.55
Cd	0.795±0.071**	76.44	0.868±0.013***	84.79
Pb	1.388±0.021***	59.81	1.611±0.015***	69.62
Cu	1.737±0.202**	81.27	1.688±0.028***	85.83
Cr	1.634±0.105**	34.00	1.141±0.069**	46.07

*** $p< 0.001$: **$p<0.01$

Fig.1a Biomass of *A.flavus* treated with different concentration of heavy metals

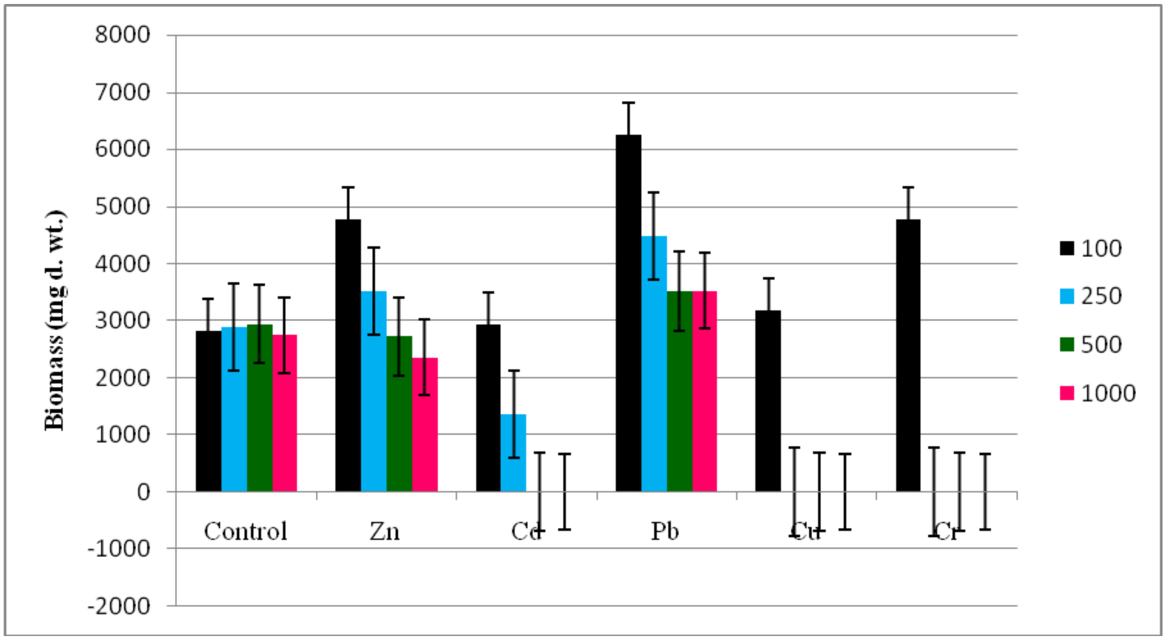


Fig.1b Biomass of *A.fumigatus* treated with different concentration of heavy metals

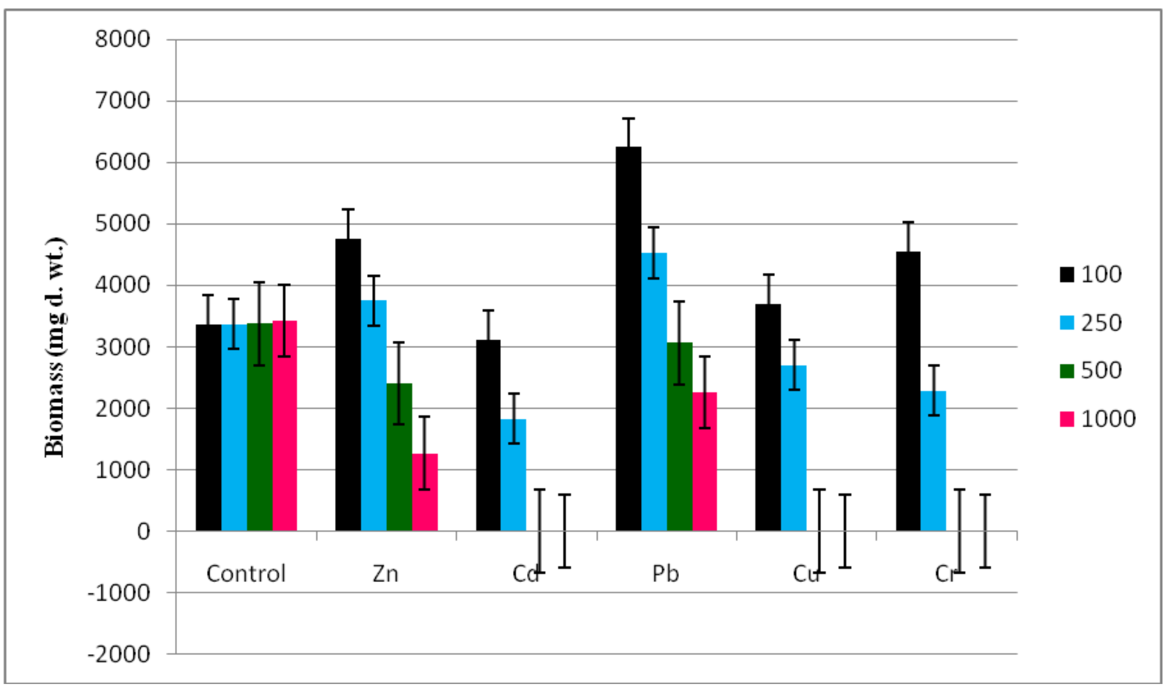


Fig.2a Accumulation of heavy metals (%) by *A.flavus*

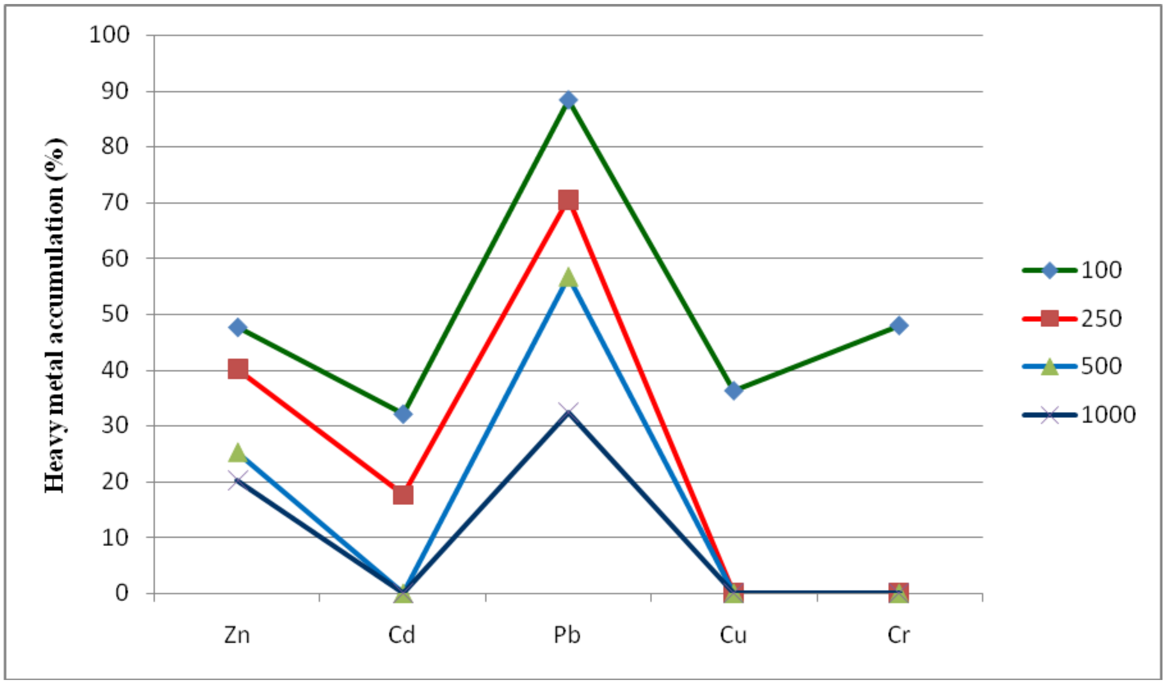


Fig.2b Accumulation of heavy metals (%) by *A.fumigatus*

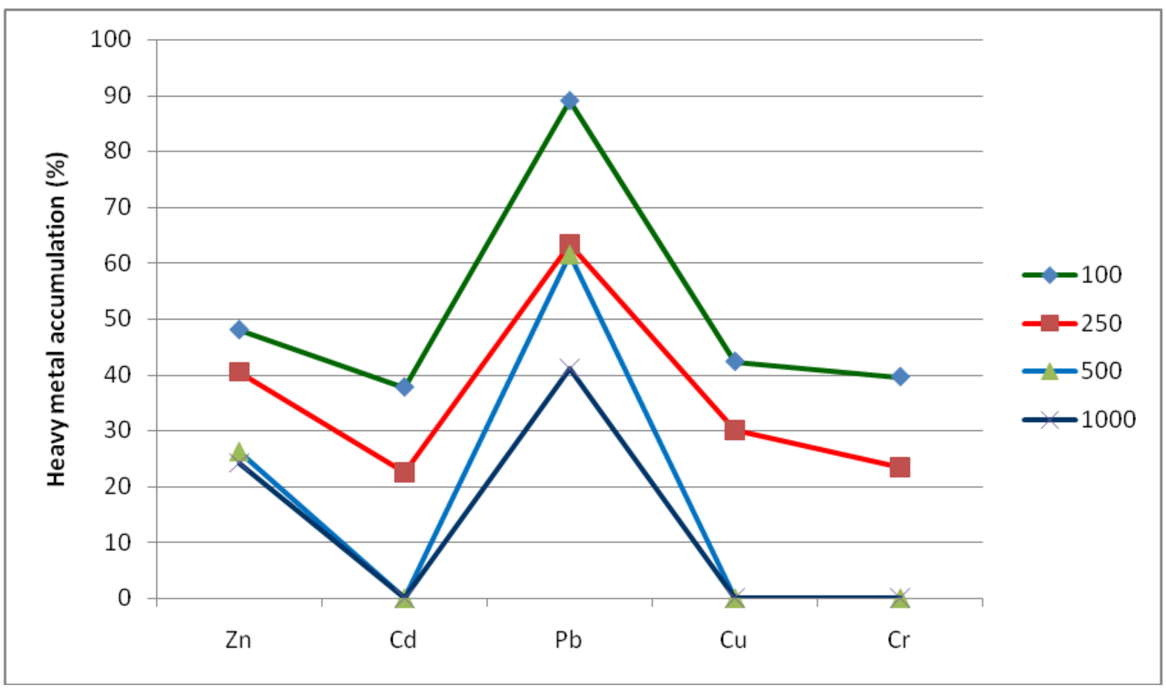


Fig.3a Effect of pH on metal uptake by *A.flavus*

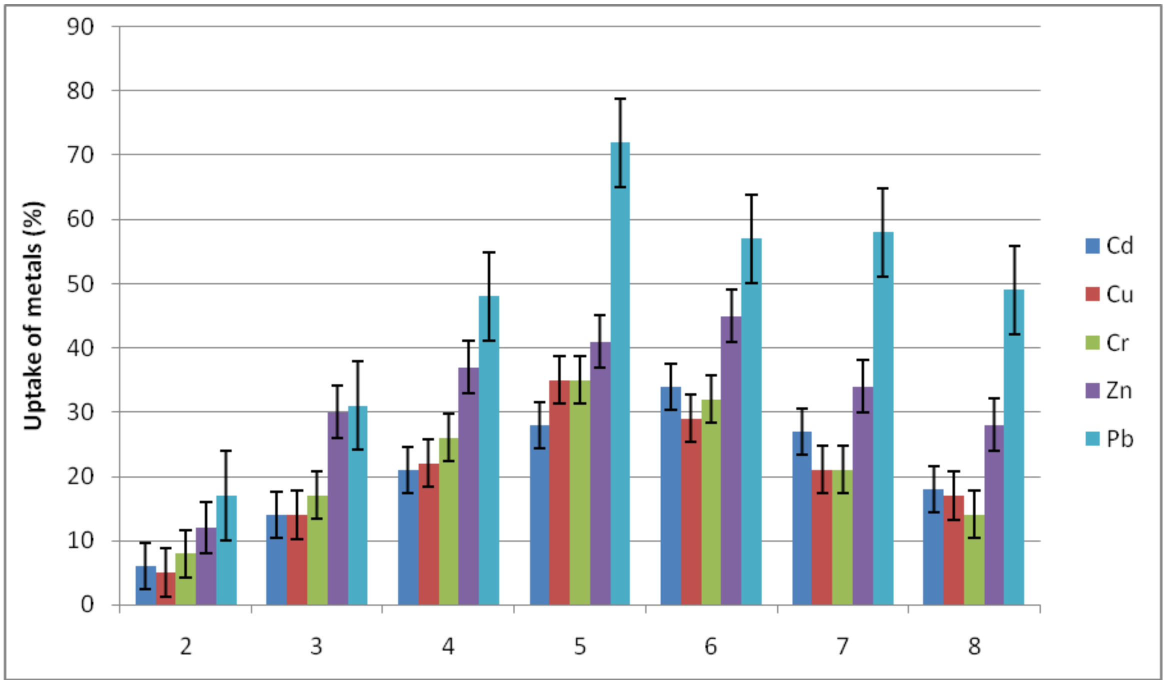


Fig.3b Effect of pH on metal uptake *A.fumigatus*

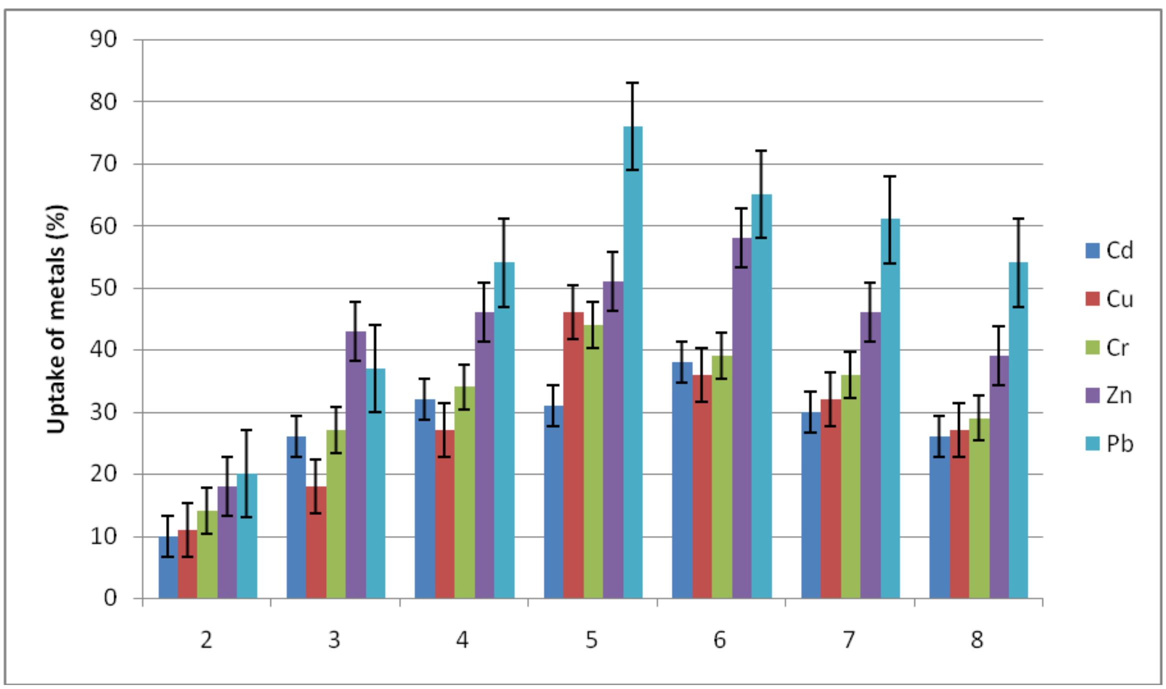


Fig.4a Effect of Temperature (^{o}C) on metal uptake *A. flavus*

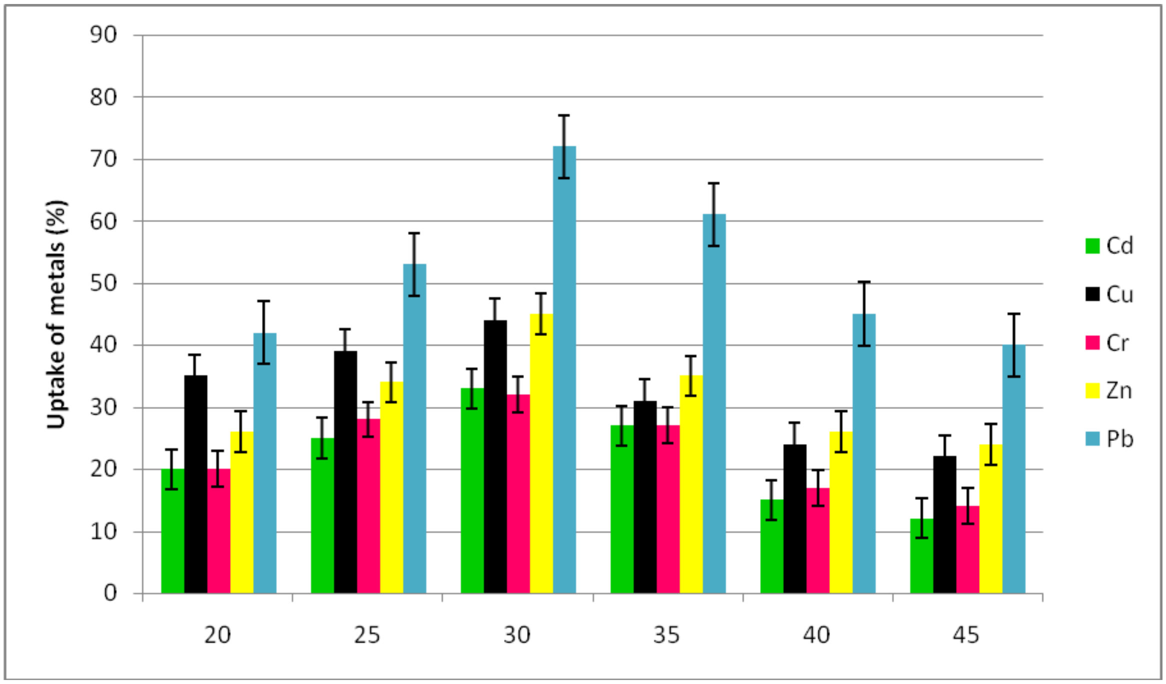


Fig.4b Effect of Temperature (^{o}C) on metal uptake by *A.fumigatus*

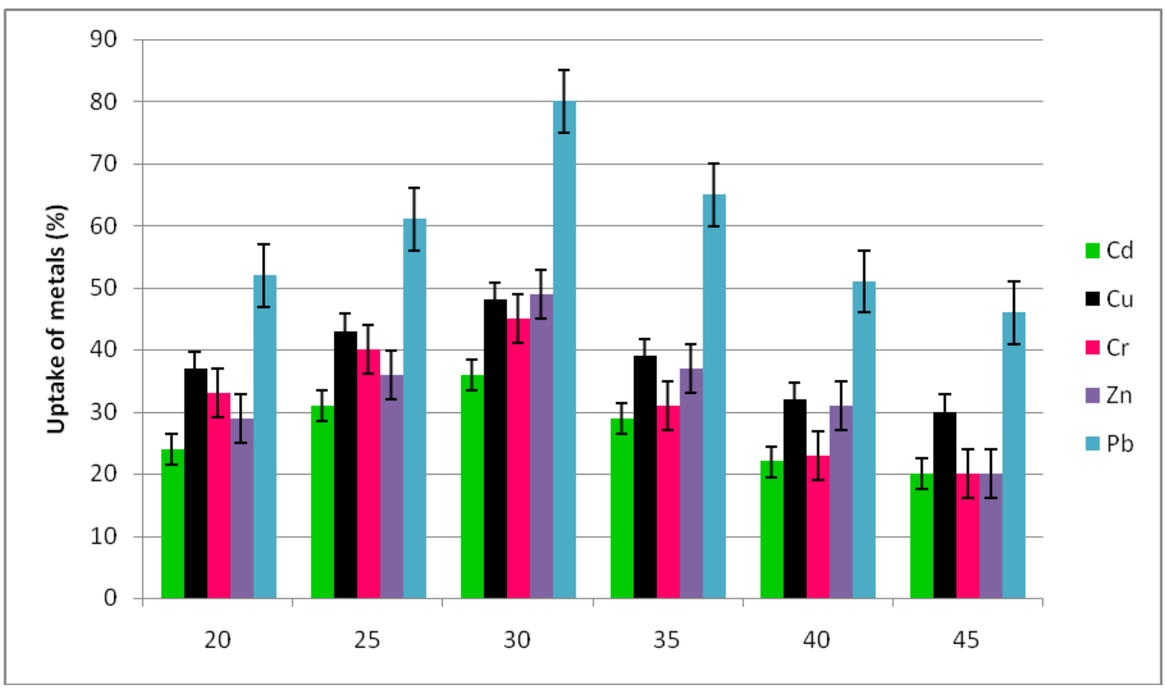


Fig.5a Effect of contact time on bioaccumulation of heavy metals by *A.flavus*

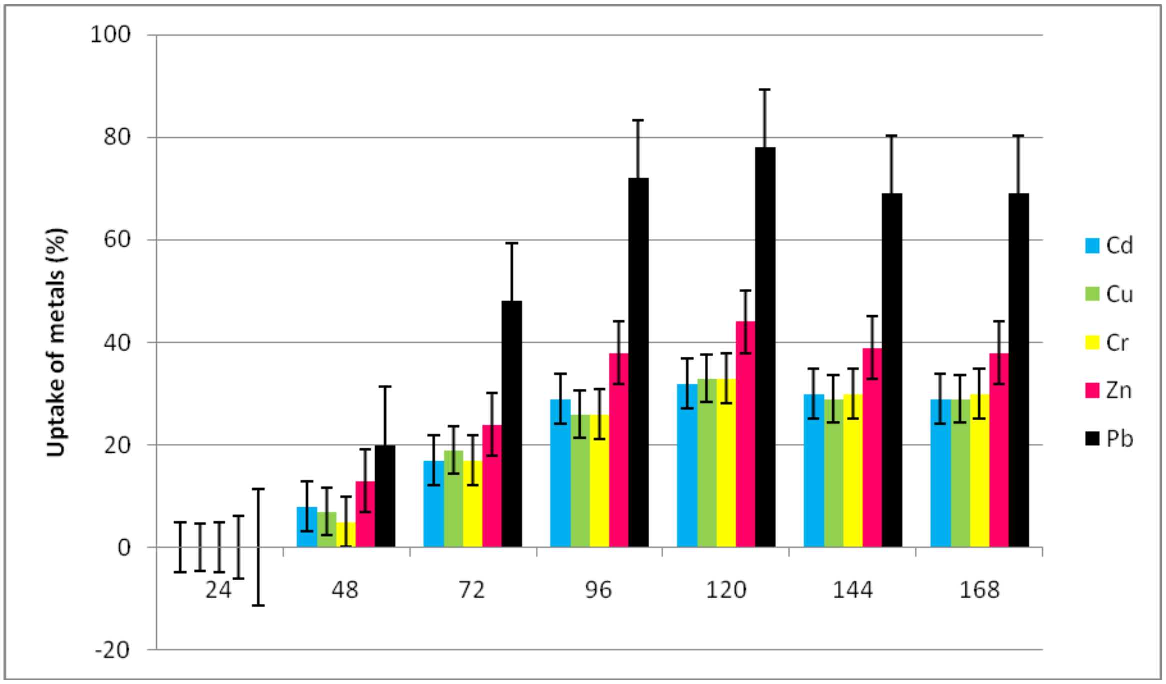
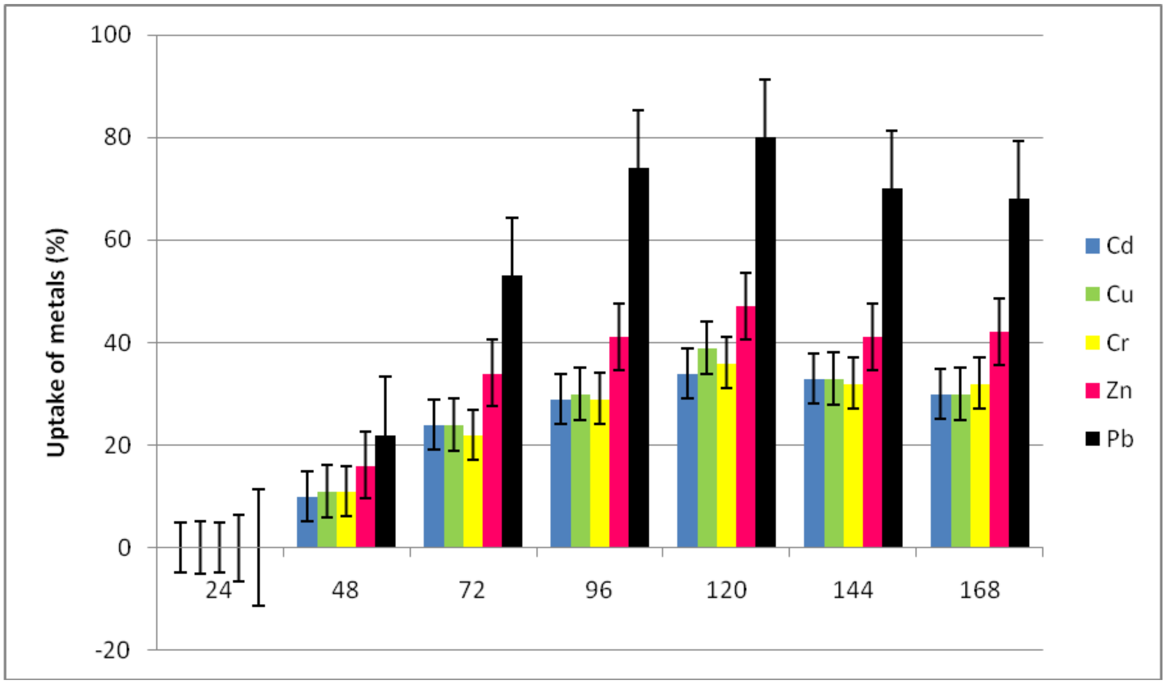


Fig.5b Effect of contact time on bioaccumulation of heavy metals by *A.fumigatus*



Effect of Temperature

Temperature is a crucial parameter in adsorption processes. The effect of temperature on the uptake of heavy metals (Zn, Cd, Pb, Cu and Cr) by *A.Flavus* and *A.fumigatus* was carried out at different temperature between 20^{o}C to 45^{o}C. It is evident (Fig: 4 a,b) that the adsorption of heavy metals by these fungal biomass increased with increase in temperature between 25^{o}C to 30^{o}C. However subsequent decrease in adsorption capacity was observed when temperature was raised by 35^{o}C.At high temperature, the attractive forces between biomass surface and metal ions are weakened and the sorption decreases which results in the decrease in the thickness of the boundary layer, due to the increased tendency of the metal ion to escape from the biomass surface to the solution phase. The similar result as decrease in adsorption as temperature increase were reported by (Horsfall and Spiff 2005).

Effect of Contact time

Bioaccumulation of heavy metals was determined at different incubation period of 24- 168 husing metal solution containing 100 mg l^{-1} concentration. It was noticed that under optimum pH and temperature, the heavy metals accumulation by *A.flavus* and *A.fumigatus* was maximum at 120h of growth period (Fig. 5a, b). After 120 h of incubation period, metal accumulation remained constant. Removal efficiency increased with an increase in contact time and this can be explained by the affinity of the adsorbents towards metal ions (Anima *et al.,* 2004).

Removal of heavy metals from untreated paper mill effluent

Microbes in metal polluted environments adapt to toxic concentration of heavy metals and become metal resistant (Prasenjit and Sumathi, 2005). In this study, two types of biosorbent of the *Aspergillus species* were used for the reduction of heavy metals from the mixture of untreated paper mill effluents collected from there sites. The results showed increasing amount of heavy metals had different influence on the fungal biomass and heavy metals accumulation. The isolated fungi indigenous to paper mill sludge were found to reduce some heavy metals at a concentration of 1-3mg/l^{-1} present in the pulp and paper mill effluent. Significant (P<0.01-0.001) reduction of Cu (81.27%), Cd (76.44%), Zn (64.95%), Mn (60.86%) and Pb (59.81%) in the presence of *A.flavus.* whereas, Significant (P<0.01-0.001) reduction of Cu (85.83%), Cd (84.79%), Zn (79.55%), Mn (71.4%) and Pb (69.62%) in the presence of *A.fumigatus*. Srivastava *et al.,* (2007) reported that industrial effluent containing toxic organic compounds inhibit the fungal growth but enhance heavy metal biosorption. Similarly in this study the results of heavy metal reduction rate was found to be higher whereas the observed biomass of *A.flavus* and *A.fumigatus* decreased and no growth was reported with increased the metal ion concentration of Cu and Cd. The removal of heavy metal efficiency of *A.fumigatus* proved superior in removal of Pb, Cd and Cu compared to *A.flavus*.

Fungi have been identified as superior candidates for metal bioremediation (Bai and Abraham, 2001). The hyper metal tolerant fungi isolated from paper mill effluent sludge has shown vigorous growth in presence of Zn^{2+}, Cd^{2+}, Pb^{2+}, Cu^{2+} and Cr^{2+} compared to control fungi without treated with metals. The fungi selected and tested in this study. *A.flavus* and *A.fumigatus* have shown unique metal accumulation and tolerance characteristics and easy to culture in sample media. The fungi exhibited high uptake ability for Pb, Zn, Cd and Cu metals even at concentration of 100 mg/L. from paper mill effluent and synthetic medium. Application of these fungi under optimum conditions such as pH, temperature, contact time and number of metal ions made increased removal of heavy metals and in turn made these fungi to use as alternative adsorbent for conventional methods of effluent treatment.

References

Akar, T. and Tunali(2006). Biosorption characteristics of *Aspergillus flavus* biomass for removal of Pb(II) and Cu(II) ions from an aqueous Solution. Biores. Technol., 97, 1780-1787.

APHA, (2005). Standard methods for the examination of water and waste water. 21st Edn., Washington DC, USA.

Arica, M. Y., Arpa, C., Ergene, A., Bayramoglu, G., Genc. O (2003). Ca-alginate as a support for Pb(II) and Zn(II) biosorption with immobilized *Phanerochaete chrysosporium*. Carbohydr. Polym. 52, 167-174.

Bai, R. S., Abraham, T. E., (2001). Biosorption of Cr(IV) from aqueous solution by *Rhizopus nigricans*. Bioresource Technology. 97, 1780-1787.

Chen, C. and J. Wang., (2007). Response of *Saccharomyces cerevisiae* to lead ion stress. Appl. Microbiol. Biotechnol., 74, 683-687.

Goel, P. K. (1996). Water pollution: Causes, effects and control. New Age International Pvt. Ltd, Publishers, New Delhi.

Juwarkar, A. (1988). Bioaccumulation of Zinc by *Penicillium* sp. Current Science. 57:251-252.

Kapoor, A. and Viraraghavan, T. (1997). Heavy metal biosorption sites in *Aspergillus niger*. Bioresour. Technol. 61, 221-227.

Kapoor, A., Viraraghavan, T. and Cullimor, D. R. (1999). Removal of heavy metals using the fungus *Aspergillus niger*, Bioresource Technology, 70: 95-104.

Leung, W. C., M. F, Wong, H. Chua, W. Lo, P.H.F. Yu and C. K. Leung. (2000). Removal and recovery of heavy metals by bacteria isolated from activated sludge treating industrial effluents and municipal waste water. Wat. Sci. Technol., 41,233-240.

Nagamani, A., I. K. Kunwar and C. Manoharachary. (2006). Hand book of soil fungi. I. K. International Pvt. Ltd., New Delhi.

Prasenjit, B. and S. Sumathi., (2005). Uptake of chromium by *Aspergillus foetidus*. J. Mater. Cycles Waste Manag. 7:88-92.

Srivastava, S., A. H. Ahmad and I. S. Thakur., (2007). Removal of chromium and pentachlorophenol from tannery effluent. Biores. Technol., 98, 1128-32.

Thippeswamy, B., Shivakumar, C.K. and Krishnappa, M., (2012). Bioaccumulation potency of *Aspergillus niger* and *Aspergillus flavus* for removal of heavy metals. Journal of Environmental Biology, 33: 1063-1068.

Verma, V. K., R. K. Gupta and J. P. N. Rai., (2005). Biosorption of Pb and Zn from pulp and paper industry effluent by hyacinth (*Eichhornia crassipes*). J. Sci. Ind. Res., 64, 778-781.

How to cite this article:

Ezhilvanan, M., S. F. Lesley Sounderraj and Nancy Lesley. 2021. Removal of Heavy Metals using the Isolates of *Aspergillus* sp. Isolated from Contaminated Pulp and Paper Mill Sludge. *Int.J.Curr.Microbiol.App.Sci.* 10(04): 890-902. doi: https://doi.org/10.20546/ijcmas.2021.1004.096