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Comparative Analysis of Oven-dried and Non-growing Strains of *Aspergillus niger* for the Removal of Lead under Laboratory Conditions

[1]Ali Abbas Qazilbash, [2]Rani Faryal, [3]Ayesha Tabasum, [3]Asima Lodhi, [3]Safia Ahmad and [3]Abdul Hameed
[1]National Expert Laboratories Development and Accreditation Support,
Trade-Related Technical Assistance Program (TRTA), United Nations Industrial Development Organization (UNIDO), P.O. Box 1051, 2nd Floor, Saudi-Pak Tower, 61-A Jinnah Avenue, Islamabad, Pakistan
[2]Department of Bio-Sciences, COMSATS Institute of Information Technology,
Islamabad Campus, # 30, Sector H-8/1, Islamabad, Pakistan
[3]Microbiology Research Laboratory, Department of Biological Sciences,
Quaid-i-Azam University, Islamabad, Pakistan

Abstract: Over the years, with the active spread and development of the industries, heavy metals, which are either used, or produced, as by-products, by numerous manufacturing, industrial, refining and mining processes, have become ubiquitous, persistent environmental pollutants. Bioremediation is a cost-effective technology that may be used to remove a variety of pollutants and contaminated sites. This study was designed to compare the capabilities of identified *Aspergillus niger* strains in the oven-dried (dead) and non-growing (living) forms to adsorb Lead (Pb) from SDB media containing the HM under optimized laboratory conditions using the shake-flask incubation method. *Aspergillus niger* showed a higher percentage of Pb adsorption in the oven-dried, as compared to the non-growing state. Furthermore, the adsorption by oven-dried strains was also high at higher concentrations of the heavy metal, whereas adsorption of Pb effectively took place at the low concentration levels for the non-growing strains. Analysis of the correlation matrices showed significant positive correlation between strains at high Pb concentrations under the oven-dried condition and significant positive correlation at low Pb concentrations under the non-growing conditions. The percentage Pb adsorption of both strains under oven-dried conditions was over 90% for the concentration range of 100-500 ppm, with the highest being 99.95% at 500 ppm for strain NP 18 after 80 min of incubation. Furthermore, for the non-growing strains this maximum adsorption was achieved for the concentration range of 100-300 ppm and that too was 96.37% achieved by strain NP 17 at 300 ppm concentration after 30 min, after which the toxic effects of the HM on the live fungal strains diminished their capacity to adsorb the metal. When comparing the two biotreatment regimens of oven-dried fungal biomass, verses the use of non-growing pellets of fungi, there is overwhelming evidence to support the use of the former as a method to removal HM contamination from polluted sites, particularly at high concentration levels.

Key words: Lead removal, oven-dried, living, *A. niger* strains

INTRODUCTION

Lead (Pb), a heavy metal produced as a by-product of fossil fuel combustion in its inorganic compound form, as well as a variety of industries and at solid waste dump sites, has a debilitating effect on the human body. Lead, even in low doses, may cause developmental disorders in fetuses, infants and the young, as well as, brain damage, behavior changes/abrupt mood swings with violent tendencies, juvenile delinquency, irritation of the respiratory tract, intoxication of the Central Nervous System (CNS), gastrointestinal complications. Lead has also been linked to permanent and possibly fatal consequences, especially in fetal and child development, leading to low birth weight, with obvious consequences for the poor-living at a lower socio-economic strata. In some cases elevated Pb levels in the blood and seminal fluids have been linked to unexplained male infertility

Corresponding Author: Dr. Ali Abbas Qazilbash, National Expert on Laboratory Development and Accreditation Support, Trade-Related Technical Assistance Program (TRTA), United Nations Industrial Development Organization (UNIDO), P.O. Box 1051, 2nd Floor, Saudi-Pak Tower, 61-A Jinnah Avenue, Islamabad, Pakistan Tel: +92 51 280 0348/2372, +92 51 265-2084 Fax: +92 51 280 0129

(Needleman *et al*., 2002; Benoff *et al*., 2003, Lévesque *et al*., 2003; Joffe *et al*., 2003; Krieger *et al*., 2003; Elliot *et al*., 2004).

Removal of heavy metal ions in substantial quantities from contaminated soils, especially industrial effluents, using fungi is well documented (Siegel *et al*., 1990; Holan and Volesky, 1995; Kapoor and Viraraghavan, 1995). Researchers have shown that *Aspergillus niger* is capable of removing heavy metals such as Pb, Cd and Cu from aqueous solutions (Kapoor and Viraraghavan, 1997). The removal of heavy metals, from wastewaters and soils, by fungi is of industrial relevance because, not only does this process clean the environment and protect its biodiversity, but also allows for the recovery the metals and their subsequent re-use (Gadd, 1986).

Fungi are well suited for this purpose of bioremediation, as they exhibit a certain degree of tolerance for adverse environmental conditions, which includes relatively high levels of metals and other toxins within specific environs, like low pH, capacity to bind heavy metals to cell walls and enhanced intracellular accumulation of these toxins (Gadd, 1986).

Living, or non-living microorganisms, or just their enzymes may be used to accomplish the task of bioremediation; a process that employees the use of specific microorganisms, following minor modifications, that remove particular contaminants from the polluted sites (Wise, 1987; Atlas and Unterman, 1999).

The possibility of employing non-living fungi to adsorb Pb was also investigated by researchers. The investigators found that in case of non-living fungal biomass, the adsorption takes place in the chitin structure of the cell (Nui *et al*., 1993; Zhang *et al*., 1998).

Biosorption, a refined method of bioremediation, involves the absorption of metal ions, by specific microorganisms, from a polluted site (Tobin *et al*., 1990). One advantage of this process is that after their physical removal from the environment, the metal ions may be recovered, stabilized, or buried, whatever is deemed as most suitable. The biological components in this process may be either living, or non-living, as the process does not require active cell metabolism (Kuhn and Pfister, 1990; Gadd and White, 1993; Volesky and Holan, 1995).

This study was designed to compare the biosorption capability of oven-dried (dead) and non-growing (living) strains of *Aspergillus niger* in the removal of Pb under optimized laboratory-scale conditions. In addition, significant correlations, between dead and living strains of *Aspergillus niger* for the removal of Pb, were determined to help in identifying the best possible method for the removal of Pb from polluted sites.

MATERIALS AND METHODS

This study was carried out at the Microbiology Research Laboratory of the Department of Biological Sciences at Quaid-i-Azam University from 2001-2003.

The oven-dried fungal biomass of both strains, *Aspergillus niger* (NP 17 and NP 18) was obtained, after growing these strains in optimized conditions as determined through the previous experiments. Each strain was then cultured in five, 500 mL flasks containing 300 mL of Sabouraud Dextrose Broth (SDB) optimized at pH 4, incubated at 40°C for 4 days. After the stipulated time, these cultures were filtered and biomass collected was washed with distilled water. Following this, the filter papers along with the biomass were placed in an oven at 55°C for the time necessary to dry the biomass, which was determined when its weight reached a constant value. After drying this biomass was milled, to approximately a 30-40 mesh size and stored, under sterile conditions, for further use in the experiments to determine its ability to adsorb metal (Pb) ions (Zhang *et al*., 1998).

The non-growing of fungal biomass of both strains was obtained in the form of pellets, 2-3 mm in diameter by growing each strain in four, 500 mL flasks, containing 100 mL of SDB at pH 4. After inoculation with both strains of the fungal isolates, the flasks were incubated at 40°C for 4 days. After which, the cultured biomass was filtered and washed with 0.1 M sodium-acetate buffer at pH 4.4 (Brady and Tobin, 1994).

Aqueous solutions of Pb salt $\{Pb(N0_3)_2\}$ of varying concentrations, i.e., 100, 200, 300, 400 and 500 mg L^{-1}, were prepared. The oven-dried milled biomass (0.4 g) of both fungal strains was added in each flask. Pellets of non-growing biomass, equal to 0.1 g dry weight, of both strains were added to a separate set of flasks. The two sets of flasks were incubated, under optimized conditions in shaker incubator for 70 min under predetermined optimized conditions. At 10 min intervals, i.e., at 0, 10, 20, 30, 40, 50, 60 and 70 min, 5 mL of the test sample from each flask was drawn and filtered by Whatmann filter paper No.1.

The filtrate was digested with HNO_3 (Clesceri *et al*., 1989) and the metal (Pb) analysis carried out using the atomic absorption spectrophotometer (Solar Unicam). Stock solution of Pb metal of 100 ppm was prepared by using the salt, $Pb(NO_3)_2$, by dissolving 0.016 g of the salt in 20 mL of distilled water. The solution was then diluted by adding more distilled water to make the volume up to 100 mL. Standard metal solutions were prepared in the optimum concentration range (2, 4 and 6 ppm) by appropriate dilution of the stock metal solution.

The appropriate hollow cathode lamp for Pb was used in this procedure and allowed to warm for a minimum of 15 min. Following this, a series of standards for Pb (2, 4 and 6 ppm) were run and a calibration curve was prepared by plotting on linear graph paper (absorbance of standards versus their concentrations). Afterwards, the analysis of diluted samples stored in plastic bottles was individually conducted, one by one. A blank was analyzed after each sample so that clear and precise data may be obtained.

Statistical analysis was carried out by computing the correlation between various aspects, parameters and experimental conditions and the significant correlations analyzed (Steel and Torrie, 1960).

RESULTS

Biosorption of Pb under oven-dried conditions: Oven-dried biomass of both fungal strains was produced by growing these strains under optimum pH and temperature. This fungal biomass was then subjected to a series of experiments to test its ability to remove Pb from the culture media containing varying concentrations of the heavy metal.

Oven-dried biomass of both strains of *A. niger* NP 17 and NP 18 were tested for their ability to absorb Pb in shake-flask incubator on media containing varying concentrations of the heavy metal.

A very distinct pattern emerged from the readings of these series of experiment. Both the fungal strains did adsorb Pb in large amounts, but followed a very particular method. At low concentrations, NP 17 adsorbed 95.52%, at 100 mg L^{-1} and 95.18% at 200 mg L^{-1}, reaching this maximum absorbance within 30 and 20 min, respectively, of its incubation in the media. However, at higher concentrations, NP 17 took longer to reach its maximum absorbance, which even though was greater in terms of numerical value, did take 40 min (99.78%) at 300 mg L^{-1} of Pb, 70 min (98.82%) and 80 min 1(97.19%) at 400 and 500 mg L^{-1} concentrations, respectively. *A. niger* NP 18 also showed rapid adsorptive qualities at low concentrations, 94.05% in 30 min of incubation with media containing 100 mg L^{-1} of Pb and at higher concentrations removed more Pb over a longer period of time 99.66% over 70 min, at Pb concentration of 300 mg L^{-1} and 99.95% in 80 min at Pb concentration of 500 mg L^{-1} (Fig. 1).

Correlation analysis between salt concentration and Pb removal by oven-dried *A. niger* strains NP 17 and NP 18, revealed that significant positive correlation ($p<0.05$) patterns existed between these two strains at identical as well as slightly variant Pb concentrations. Strains NP 17 and 18 showed significant positive correlation in media with Pb concentrations of 200, 300 and 400 mg L^{-1}. Strain NP 17 at Pb concentration of 300 mg L^{-1} also displayed positive correlation with strain NP 18 at Pb concentrations of 400 mg L^{-1} and with NP 17 at Pb concentrations of 500 mg L^{-1}. Strain NP 18 at Pb concentration of 300 mg L^{-1} showed positive correlation with strain NP 17 and NP 18 at 400 mg L^{-1} concentration of Pb, with NP 17 and NP 18 at 500 mg L^{-1} of Pb. Strain NP 17 at Pb concentration of 400 mg L^{-1} showed positive correlation with strain NP 17 and NP 18 at 500 mg L^{-1} concentration of Pb. Strain NP 18 at 400 mg L^{-1} of Pb showed positive correlation with strains NP 17 and NP 18 at 500 mg L^{-1} of Pb (Fig. 2-5).

Biosorption of Pb by non-growing fungal biomass: The objective of study was to investigate the biosorptive abilities of living, but non-growing fungal biomass.

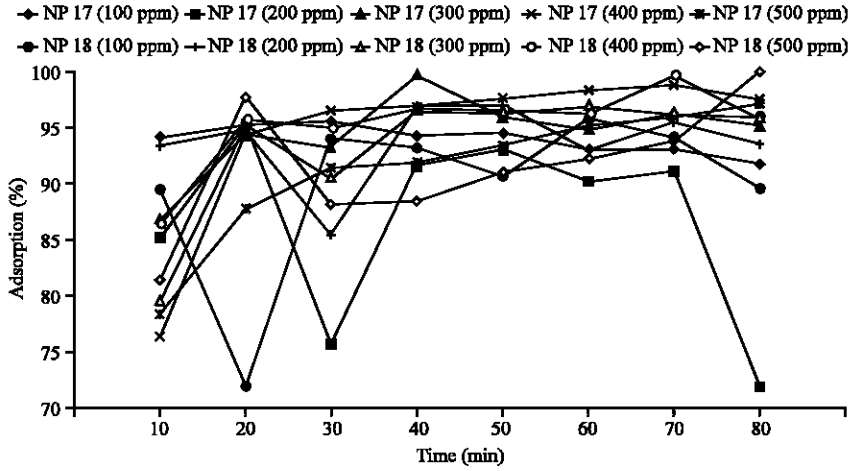

Fig. 1: Effect of metal concentration (ppm) on adsorption of Pb by oven-dried Aspergillus niger NP 17 and NP 18 under optimum conditions

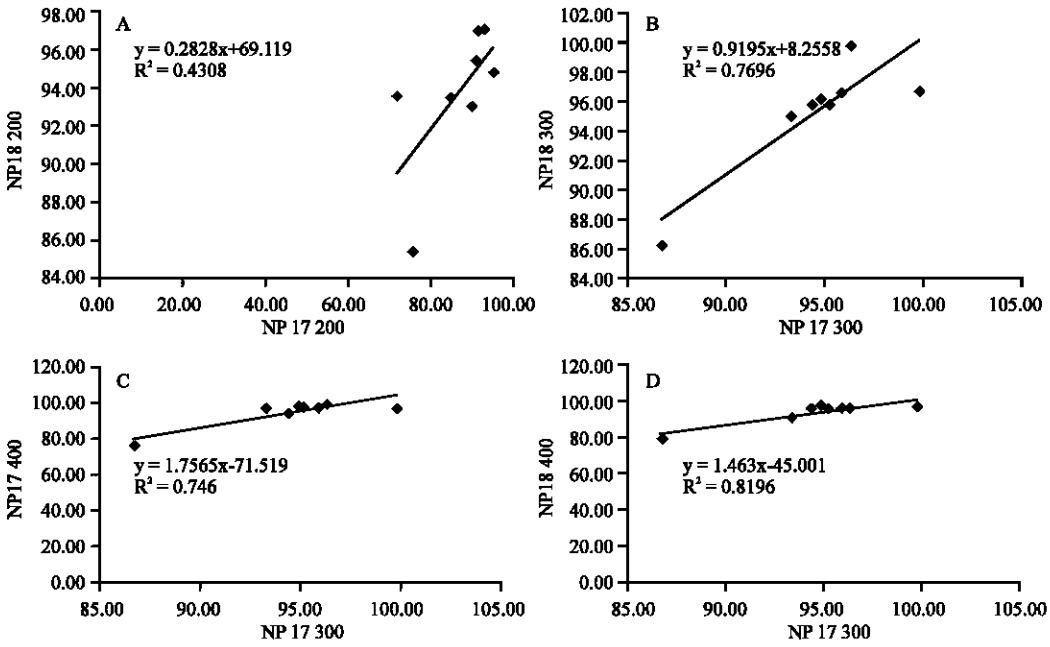

Fig. 2: Significant positive correlation ($p<0.05$) between metal concentrations (ppm) and adsorption of Pb (%) by oven-dried *Aspergillus niger* NP 17 and NP 18 under optimum conditions, showing positive correlation of strains NP17 and NP18 at the rate of 200 ppm (A) and strain NP17 at the rate of 300 ppm with NP18 at the rate of 300 ppm (B), with NP17 and NP18 at the rate of 400 ppm (C and D)

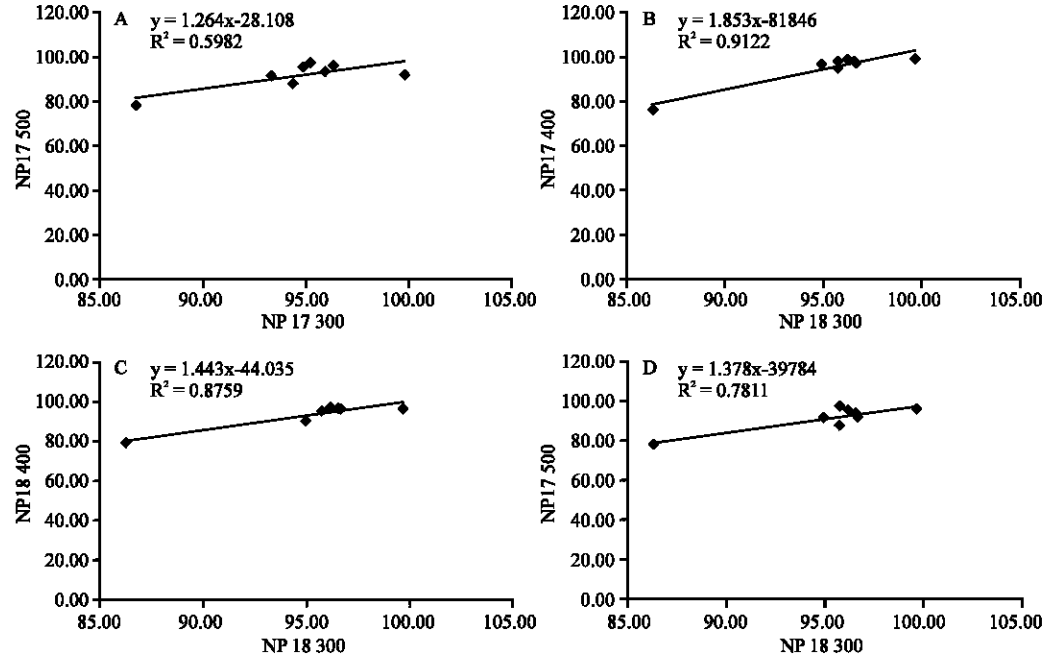

Fig. 3: Significant positive correlation ($p<0.05$) between metal concentrations (ppm) and adsorption of Pb (%) by oven-dried *Aspergillus niger* NP 17 and NP 18 under optimum conditions, showing positive correlation of strain NP17 at the rate of 300 ppm with NP18 at the rate of 500 ppm (A) and strain NP18 at the rate of 300 ppm with NP17 and NP18 at 400 ppm (B and C) and NP17 at the rate of 500 ppm (D)

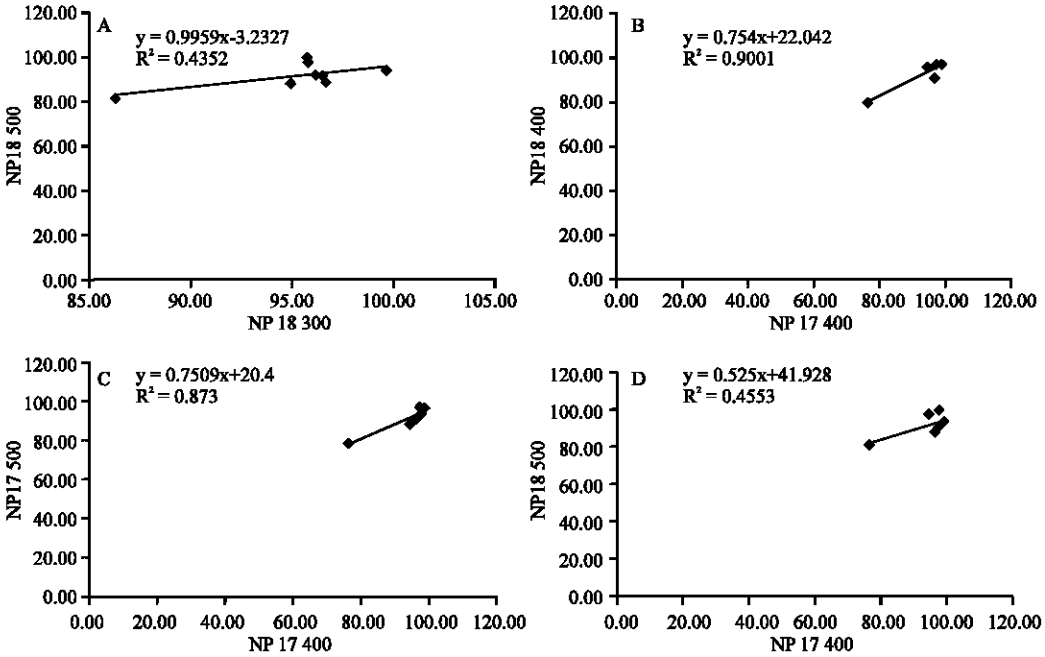

Fig. 4: Significant positive correlation ($p<0.05$) between metal concentrations (ppm) and adsorption of Pb (%) by oven-dried *Aspergillus niger* NP 17 and NP 18 under optimum conditions, showing positive correlation between strain NP18 at the rate of 300 ppm and NP18 at the rate of 500 ppm (A) and between strain NP17 at the rate of 400 ppm with NP18 at the rate of 400 ppm (B) and NP17 and NP18 at the rate of 500 ppm (C and D)

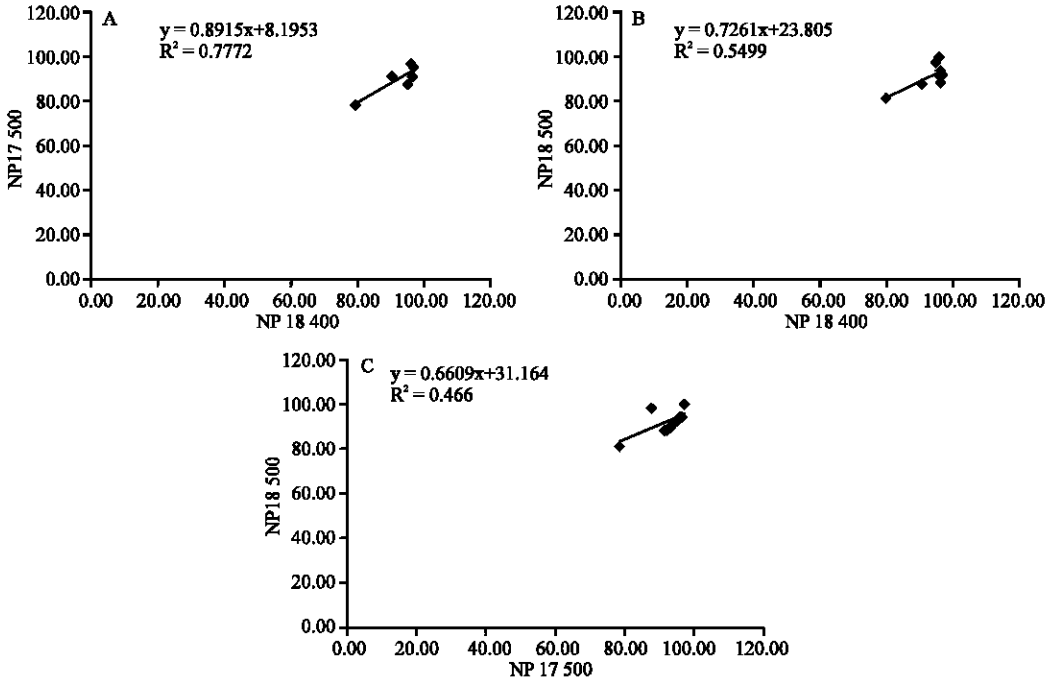

Fig. 5: Significant positive correlation ($p<0.05$) between metal concentrations (ppm) and adsorption of Pb (%) by oven-dried *Aspergillus niger* NP 17 and NP 18 under optimum conditions, showing positive correlation between strain NP18 at the rate of 400 ppm with NP17 at the rate of 500 ppm (A) and NP 18 at the rate of 500 ppm (B) and between strain NP 17 at the rate of 500 ppm with NP18 at the rate of 500 ppm (C)

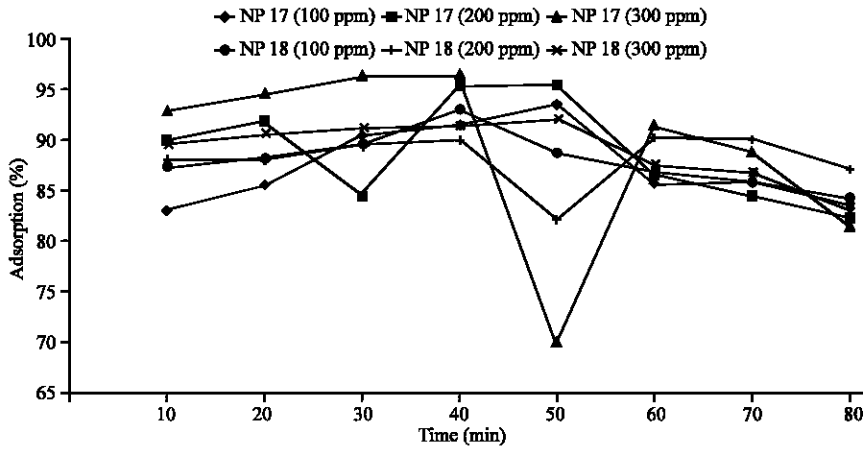

Fig. 6: Effect of mental concentration (ppm) on the adsorption of Pb by non-growing biomass of *Aspergillus niger* strains NP 17 and 18 under optimum conditions

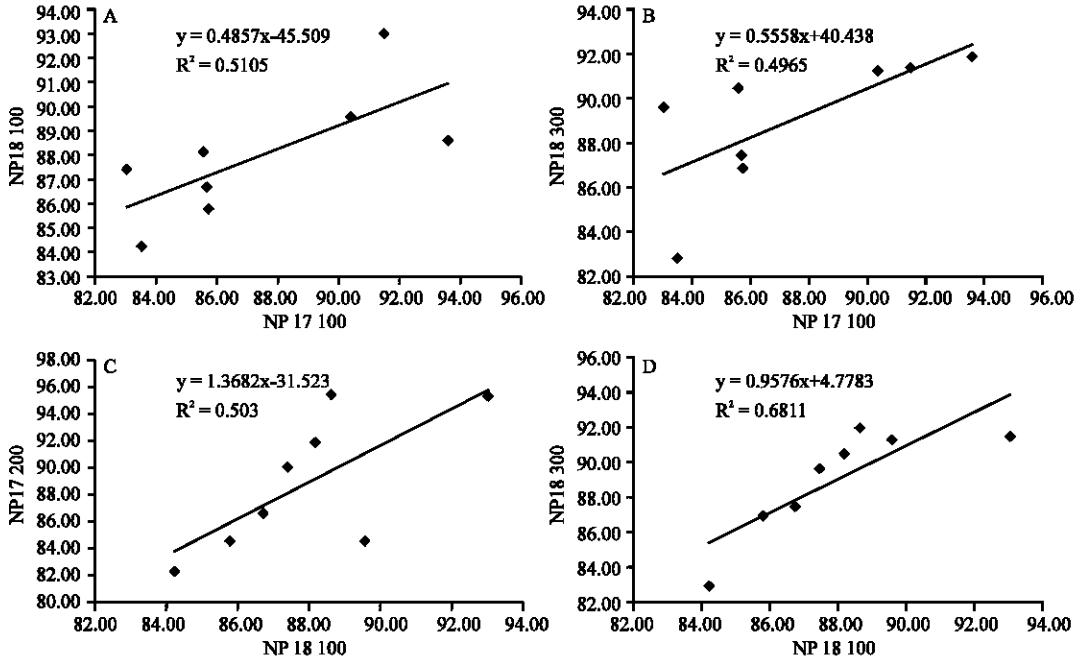

Fig. 7: Significant positive correlation ($p<0.05$) between mental concentration (ppm) and adsorption of Pb (%) by non-growing biomass of *Aspergillus niger* strains NP 17 and 18 under optimum conditions, showing correlation of strain NP17 at the rate of 100 ppm with NP18 at the rate of 100 ppm (A), with NP18 at the rate of 300 ppm (B) and strain NP18 at the rate of 100 ppm with NP17 at the rate of 200 ppm (C) and with NP18 at the rate of 300 ppm (D)

Initially, a set of experiments was carried out to determine the optimum pH and temperature for maximum Pb absorption. After determining the optimum conditions, the non-growing pellets of the two isolates, prepared as described in the previous chapter were cultured in shake-flask incubator, on SDB, with varying concentrations of Pb, under optimum conditions to ascertain the ability of both the fungal strains, to bio-absorb Pb, in this particular form. The pH of the media was adjusted to 4 and temperature of 40°C was selected as the incubation temperature, for the first set of experiments.

Following these determinants, the last series of experiments, for this part of the study, were carried out to determine the maximum heavy metal absorbency of these two fungal strains in the non-growing fungal biomass form. These isolates were tested against three different Pb concentrations, i.e., 100, 200 and 300 mg L^{-1}, for metal absorbency.

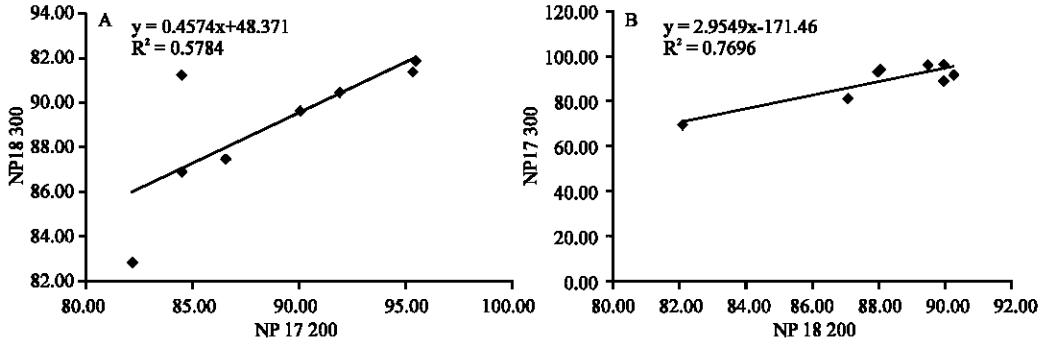

Fig. 8: Significant positive correlation ($p<0.05$) between mental concentration (ppm) and adsorption of Pb (%) by non-growing biomass of *Aspergillus niger* strains NP 17 and 18 under optimum conditions, showing correlation of strain NP17 at the rate of 200 ppm with NP18 at the rate of 300 ppm (A) and strain NP18 at the rate of 200 ppm with NP17 at the rate of 300 ppm (B)

The findings of these series of experiments presented a comparative analysis with adsorption abilities of oven-dried fungal biomass. From these readings, apparently, it seems that fungal biomass in the oven-dried form is better at removing Pb from the contaminated environment. For non-growing *niger* strain NP 17, removed 93.57% of the Pb at concentration of 100 mg L^{-1}, within 50 min of incubation, however, in the oven-dried form it took just 30 min to remove 95.52% of Pb in the same concentration.

The same pattern was seen for the higher concentrations of Pb, in that NP 17 strain removed 96.37% (at 300 mg L^{-1}) of the metal within 40 min. Whereas, the same strain the oven-dried form removed 99.78% of the metal within 40 min of incubation, at the same Pb concentration. The NP 18 strain as well, in that it too absorbed less of the Pb when cultured in the non-growing fungal biomass, than when in the oven-dried state. Non-growing biomass of *niger* NP 18 absorbed 93.03% of the Pb (at 100 mg L^{-1}), within 40 min of incubation, whereas at the same concentration the very same strain in the oven dried form, adsorbed 94.05% within 30 min of incubation and adsorbed 99.78%, after 40 min of incubation at concentration of 300 mg L^{+}. This was significantly greater removal of metal than by the non-growing NP 18 form that absorbed a maximum of 91.93% Pb from media of equal concentration (Fig. 6).

The difference is more pronounced when analyzing the net absorbency, after the stipulated time to account for desorption. After 80 min of incubation, the NP 17 strain in the non-growing form absorbed 83.5, 82.21 and 81.48% Pb from concentrations of 100, 200 and 300 mg L^{-1}, respectively. Whereas, the same strain in the oven-dried biomass form adsorbed 89.6, 93.52 and 95.72% of Pb from identical media concentrations.

Correlation analysis between metal concentrations and Pb removal by non-growing biomass of *A. niger* strains NP 17 and NP 18 under optimum conditions showed an opposite trend to that computed for oven-dried fungal biomass. This was evident from the fact that, for non-growing fungal biomass, significant ($p<0.05$) positive correlation were found between these two strains at comparatively lower Pb concentrations than for the oven-dried experiments. Strain NP 17 grown in media with 100 mg L^{-1} of Pb showed positive correlation with NP 18 grown in media with 100 and 300 mg L^{-1} of Pb. While NP 18 grown in 100 mg L^{-1} of Pb showed positive correlation with NP 17 from 200 mg L^{-1} and NP 18 from 300 mg L^{-1} of Pb. As for the strains grown in 200 mg L^{-1} of Pb, NP 17 showed positive correlation with NP 18 grown in 300 mg L^{-1} of Pb and NP 18 was positively correlated with NP 17 also grown in 300 mg L^{-1} of Pb (Fig. 7 and 8).

DISCUSSION

Aspergillus niger has been shown in this study to be resistant to Lead (Pb) and that two specific strains of *A. niger* NP 17 and NP 18 showed the highest tolerance against Pb when cultured in SDA amended with this particular heavy metal. *A. niger* NP 17 showed maximum resistivity level up to 15,000 mg L^{-1}, while *A. niger* NP 18 showed even higher tolerance to Pb, growing at levels as high as 18,000 mg L^{-1}. On the basis of Minimal Resistance Levels (MRL) these strains were selected for further experiments to determine their bioremediation efficacy of Pb, particularly under oven-dried and non-growing (living) conditions, for comparative analysis. This is in agreement with findings reported by other researchers in terms of high tolerance levels of *A. niger* to HM and long-chain hydrocarbons (Sayer *et al*., 1995; Kapoor and Viraraghavan, 1997; Micheal *et al*., 2001).

The two identified strains of *Aspergillus niger*, namely: NP 17 and NP 18, were used to determine their Pb biosorption ability for a comparative analysis of the biosorptive abilities of oven-dried (dead) and non-growing pellets (living) of fungal biomass. The spherical

mycelial pellets, 2-3 mm in diameter, of non-growing biomass of both strains (NP 17 and NP 18) were produced. The pellets of both strains of *A. niger* were used to determine the percentage adsorption of Pb from solution, to compare their absorptive prowess with that of oven-dried (dead) fungal biomass (Treen-Sear *et al*., 1984; de Rome and Gadd, 1991).

Both strains of *Aspergillus niger* showed very high percentage of Pb adsorption in the oven-dried, as compared to the non-growing state. Furthermore, the adsorption by oven-dried strains was also high at higher concentrations of the heavy metal, whereas adsorption of Pb effectively took place at the low concentration levels for the non-growing strains. This was exemplified in the correlation matrices, where the comparison showed significant positive correlation between strains at high Pb concentrations under the oven-dried condition and significant positive correlation at low Pb concentrations under the non-growing conditions (Fig. 2 and 4).

Numerous researchers have also reported similar findings, where dead microbial biomass, including mycelium was found to adsorb more HM ions, often in considerably larger quantities, than living biomass (Brady and Tobin, 1994). This may be explained by the use of dead over live biomass simply because the metal removal system is not subjected to the toxicity of the adsorbed metal ions, hence dead fungal biomass has a greater tolerance, particularly at high concentrations of the contaminant. In addition, these adsorbed HM ions can be easily desorbed and the biomass can be reused rendering the dead biomass treatment system the most effective bioremedial model in use today (Kapoor and Viraraghavan, 1997).

From the study under discussion it is evident that adsorption of Pb by both strains of oven-dried (dead) *A. niger* was considerably greater across a wider range of contaminant concentrations and over a longer period of time as compared to the stains under non-growing (living) conditions. Similar findings have also been reported by other researchers (Fogarty *et al*., 1990; de Rome and Gadd, 1991; Pumpel and Schinner, 1993; Sag *et al*., 1995; Wong *et al*., 2000).

When comparing the two biotreatment regimens in this portion of the study, i.e., use of oven-dried fungal biomass, versus the use of non-growing pellets of fungi, there is overwhelming evidence to support the use of the former as a method to removal HM contamination from polluted sites, be it effluents or soil. One may argue that under optimum conditions, which may be said are different for both regimens, there is nor much difference between the two methods of biotreatment. However, upon closer examination, it may be equally arguable that the amount of HM ions removed, within the specific time frame and in higher concentrations of the contamination, the process that employs oven-dried fungal biomass is more effective.

This process also allows for desorption and therefore may be re-used over a longer period of time. This would also allow for the retrieval of the HM and reduce the expenses of having to purchase more for the specified industrial processes. Furthermore, the proliferation of living fungi may occur with the passage of time, despite these pellets being labeled as being 'non-growing'. The added advantage of using oven-dried-dead-fungal biomass, is that very little optimization is required and these strains may be used in areas where the pollutant contamination is high (Brady and Tobin, 1994). In such cases, if non-growing pellets are used, the strains would need to be genetically engineered and the microenvironment made more conduce for effective remedial of the contaminant, which would lose its cost-effectiveness and be more labor intensive (Kapoor and Viraraghavan, 1997).

The use of oven-dried (dead) fungal biomass may, therefore, indeed be labeled as the panacea for the removal of toxic substance from the environment. Not only is it cost-effective, but intrinsic bioremediation relies principally on the indigenous microorganisms, isolated from the contaminated site itself, to wield their natural power and remedy the situation. Such technologies, although not new, but certainly are in their infancy in this part of the world and must be encouraged and promoted by all concerned sectors.

As a result there have been large scale movements and changes in policy that have resulted in the complete abandonment of leaded fuels it the west the replacement of Pb in paint with less toxic supplements (Elliot *et al*., 1999). Improved industrial hygiene and increased use of new technologies to remove Pb from the environment have been actively adopted in the developed nations and must be strongly considered and implemented in developing nations as well (Khwaja, 2003).

Using fungal biomass, particularly that of oven-dried (dead) fungal strains of *Aspergillus niger*, a ubiquitous microbe, may help to reduce this HM from our environment. Not only would we be saving our children and the many generations to come from exposure to this poison, but we would also help to save the biodiveristy within a defined biosphere.

REFERENCES

Atlas, R.M. and R. Unterman 1999. Bioremediation. In: Manual of Industrial Microbiology and Biotechnology 2nd Edn., American Society for Microbiology Press, Washington DC., pp: 666-681.

Benoff, S., G.M. Centola, C. Millan, B. Napolitano, J.L. Marmar and I.R. Hurley, 2003. Increased seminal plasma lead levels adversely affect the fertility potential of sperm in IVF. Human Reprod., 18: 374-383.

Brady, D. and J.M. Tobin, 1994. Adsorption of metal ions by *Rhizopus arrhizus* biomass: Characterization studies. Enz. Microb. Technol., 16: 671-675.

Clesceri, L.S., A.E. Greenberg and R.R. Trussel, 1989. Standard Methods for the Examination of Water and Wastewater 17th Edn., APHA, AWWA, WPCF, Washington.

de Rome, L. and G.M. Gadd, 1991. Use of pellet and immobilized yeast and fungal biomass for heavy metal and radionuclide recovery. J. Ind. Microbiol., 7: 97-154.

Elliot, P., R. Arnold, D. Barltrop, I. Thornton, I.M. House and J.A. Henry, 1999. Clinical lead poisoning in England: An analysis of routine sources of data. Occup. Environ. Med., 56: 820-824.

Elliot, M.R., Y. Wang, R.A. Lowe and P.R. Kleindorfer, 2004. Environmental justice: Frequency and severity of US chemical industry accidents and the socioeconomic status of the surrounding communities. J. Epidemiol. Comm. Health, 58: 24-30.

Fogarty, R.V., P. Dostalek, M. Patzak, E. Tel-Or and J.M. Tobin, 1990. Metal Removal by Immobilized and Non-immobilized *Azolla filiculoides*. Biotechnol. Technol., 13: 533-538.

Gadd, G.M., 1986. Fungal Responses Towards Heavy Metals. In: Microbes in Extreme Environments, Academic Press, London, pp: 83-110.

Gadd, G.M. and C. White, 1993. Microbial treatment of metal pollution. Trends Biotechnol., 11: 353-359.

Holan, Z.R. and B. Volesky, 1995. Accumulation of cadmium, lead and nickel by fungal and wood biosorbents. Applied Biochem. Biotechnol., 53: 133-146.

Joffe, M., L. Bisanti, P. Apostoli, P. Kiss, A. Dale, N. Roeleveld, M.L. Lindbohm, M. Sallmén, M. Vanhoorne, J.P. Bonde and Asclepios, 2003. Time to pregnancy and occupational lead exposure. Occup. Environ. Med., 60: 752-758.

Kapoor, A. and T. Viraraghavan, 1995. Fungal biosorption-an alternative treatment option for heavy metal bearing wastewaters: A review. Biores. Technol., 53: 195-206.

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

Khwaja, M.A., 2003. Pollution of the River Kabul. In: The Politics of Managing Water, Oxford University Press, Karachi, Pakistan, pp: 27-30.

Krieger, N., J.T. Chen, P.D. Waterman, M.J. Soobader, S.V. Subramanian and R. Carson, 2003. Choosing area based socioeconomic measures to monitor social inequalities in low birth weight and childhood lead poisoning: The public health disparities geocoding project (US). J. Epidemiol. Comm. Health, 57: 186-199.

Kuhn, S.P. and R.M. Pfister, 1990. Accumulation of cadmium by immobilized *Zooloae ramigera* 115. Ind. Mcriobiol., 6: 123-128.

Lévesque, B., J.F. Duchesne, C. Gariépy, M. Rhainds and P. Dumas *et al*., 2003. Monitoring of umbilical cord blood lead levels and sources assessment among the Inuit. Occup. Environ. Med., 60: 693-695.

Micheal, S.P., J.J. Classen and G.A. Payne, 2001. *Aspergillus niger* absorbs copper and zinc from swine wastewater. Biores. Technol., 77: 41-49.

Needleman, H.L., C. McFarland, R.B. Ness, S.E. Fienberg and M.J. Tobin, 2002. Bone lead levels in adjudicated delinquents: A case control study. Neurotoxicol. Teratol., 24: 711-717.

Nui, H., X. Xu, J.H. Wang and B. Volesky, 1993. Removal of lead from aqueous solutions by *Pencillium* biomass. Biotechnol. Bioeng., 42: 785-787.

Pumpel, T. and F. Schinner, 1993. Native fungal pellets as a biosorbent for heavy metals. FEMS Mcirobiol. Rev., 11: 159-164.

Sag, Y., D. Oeer and T. Kutsal, 1995. A comparative study of the biosorption of Pb^{2+} ions to *Z. ramigera* and *R. arrhizus*. Process Biochem., 30: 169-174.

Sayer, J.A., S.B. Raggett and G.M. Gadd 1995. Solubilization of insoluble metal compounds by soil fungi: Development of a screening method for solubilizing ability and metal tolerance. Mycol. Res., 99: 987-993.

Siegel, S.M., M. Galun and B.Z. Siegel, 1990. Filamentous fungi as metal biosorbents: A review. Water Air Soil Poll., 53: 335-344.

Steel, R.G.D. and J.H. Torrie, 1960. Sampling from a Normal Distribution. In: Principles and Procedures of Statistics with Special Reference to Biological Sciences, McGraw-Hill Book Company Inc., New York, pp: 49-66.

Tobin, M.J., D.G. Cooper and R.J. Neufeld, 1990. Investigation of mechanism of metal uptake by denatured *NPizopus arNPizus* biomass. Enz. Microbial. Technol., 12: 591-595.

Treen-Sears, M.E., B. Volesky and R.J. Neufeld, 1984. Ion exchange/complexation of the uranyl ion by *Rhizopus* biosorbent. Biotehcnol. Bioeng., 26: 1323-1329.

Volesky, B. and Z.R. Holan, 1995. Biosorption of heavy metals. Biotechnol. Prog., 11: 235-250.

Wise, D.L., 1987. Industrial Wastes. In: Bioenvironmental Systems, CRC Press, Inc., Boca Raton, Florida, 3: 44-53.

Wong, J.P.K., S.Y. Wong and N.F.Y. Tam, 2000. Nickel biosorption of two *Chorella* species, *C. vulgaris* (a commercial species) and *C. miniata* (a local isolate). Biores. Technol., 73: 133-137.

Zhang, L., L. Zhao, Y. Yu and C. Chen, 1998. Removal of lead from aqueous solution by non-living *Rhizopus nigricans*. Water Res., 32: 1437-1444.