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Bioremediation of Heavy Metals Using Isolates of Filamentous Fungus *Aspergillus fumigatus* Collected from Polluted Soil of Kasur, Pakistan

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Abstract

Microorganism executes major role in heavy metals biosorption from polluted soil and water. Heavy metals having relatively high density are toxic at low concentration. The concentration of heavy metals is increasing due to rapid industrialization. To control metal pollution biotechnology is being applied and biosorption is one of the processes of biotechnology. The study was conducted on the various isolates of highly tolerate filamentous fungal species, Aspergillus fumigatus isolated from polluted soil collected from Kasur district, Pakistan. Biosorption capacity of Aspergillus fumigatus was investigated against metals viz. lead (Pd), chromium (Cr), cadmium (Cd), nickel (Ni), copper (Cu) and zinc (Zn) at constant pH 5 and temperature 30 ºC and at 200ppm, 400ppm, 600ppm and 800ppm metal solution concentrations. The highest biosorption value (76.07) exhibited by A. fumigatus isolate K3 against Pb, followed by Cu (69.6) and Cr (40.0) at 800ppm metal concentration. The purpose of the present investigation was to investigate different fungal isolates absorption behavior towards various heavy metals toxic and detrimental to flora and fauna. The knowledge of present study will be helpful for further assessment and management of natural biosorbent (fungus) which could serve as an economical source of treating industrial effluents with toxic metallic ions.

Keywords: Biosorption, heavy metals, fungus, *Aspergillus fumigatus,* soil pollution.

Introduction

Environmental pollution is nothing but a misplaced resource; it is truer in context of the heavy metal ions. Environmental pollution due to toxic heavy metals is the major issue of the twenty first century. Heavy metals such as iron, manganese, mercury, lead, zinc, cadmium, uranium, chromium and several others are cornerstones of human progress; they are quite literally the pillars of all the major civilizations, past and present because they are used widely as part of materials construction, agriculture, transportation, and in processing of many industrial materials and commercial products[1].

With the rapid development of many industries such as mining[2], smelting procedures[3] and agriculture[4] as well as from natural activities, heavy metals have been discharged into the environment as a result of anthropogenic processes. Chemical and metallurgical manufacturing are the main sources of metal ions in the environment[5]. Heavy metals present in contaminated soil may pose a threat to human health if these metals enter into the food chain.

In the recent years, different methods are being utilized for the removal of heavy metal ions from the aqueous disposed such as ion exchange, precipitation (Chemistry), membrane technologies, electrochemical treatments, activated carbon adsorption, etc[6]. However, electrochemical treatment and precipitation (Chemistry) are deactivated on decreasing metal ion concentrations upto 50 mg/l in the aqueous solution. Therefore, each of these procedures has some demerits that exceed to merits. By applying biotechnological tools like biosorption in managing and removal of metal ions pollution has been paid much consideration and gradually becomes important technique from the last few decade[7]. Biosorption is a process of bioremediation of heavy metals by utilizing some natural biological sources including bacteria, fungi, yeast, algae, etc. In the beginning, the studies have been focus completely on the toxicological features of biosorption technique. In recent times, attempts are being made to connect biosorption phenomenon into a method for the detoxification of industrial effluents containing metallic ions by eradication or/and ultimately also metals recovery[8]. The advantage of biosorption is not only to be functioned under a broad spectrum of conditions like pH, temperature etc. but also to be found economically feasible due to the cheap raw supplies that can be utilized as biosorbents[9].

Biosorption capacity may vary extensively and mainly it is depending upon the metal ions and biosorbent involved in the processes, the use of denatured biomass can be of great concern. With these dead biomasses, heavy metal ions were clearly accumulated on cell walls, while no specific molecules were found as particular sites for metal chelating. The metal recovery and biomass production is considered as essential to get rid of metals toxicity for microbial growth, or suppression of metal addition through nutrients or excreted metabolites[10].

Filamentous fungi are employed in fermentation industries to generate diverse metabolites for exampleantibiotics, enzymes, etc. The fungi showed a great affinity for metal ions as compared to other microbes. These can accumulate metals by means of biological and physiochemical mechanisms from their external environment[11,12]. All filamentous fungi belonging to Zygomycetes group[13], in addition to small quantity of protein, contain large amounts of polymer of *N*-acetyl, chitin and chitosan, and deacetylated glucose-amine on their cell wall. Therefore, large amounts of potential binding sites are showed by free hydroxyl groups, amineand carboxyl. The amine group containing nitrogen atom and the hydroxyl group containing oxygen atom have ability to bind a proton or a metal ion, respectively, followthe electron pair sharing phenomenon. However, the electro-negativity of oxygen is higher than that of nitrogen; therefore a lone pair of electrons donated from the nitrogen will be more facile than that of the oxygen atom in the formation of metal complex[14].

In the present study, biosorption efficiency of filamentous fungus, *Aspergillus fumigatus* isolates collected from the peri-urban agricultural soil of Kasur district, towards remediation of heavy metals viz. Cu, Pb, Cr, Ni, Zn and Cd polluted this area, was evaluated by characterizing the bioaccumulation of these metals.

Material and Methods

Sample Collection and Fungus Isolation: For present study, soil samples were collected from area near to peri-urban agricultural land, Kasur district (figure-1). From selected soil samples pure culture of *Aspergillus fumigatus* isolates was isolated by soil dilution method (and preserved for further detailed investigation of heavy metal biosorption analysis. The study was conduct at the laboratory of Mycology and Ecotoxicology, Fatima Jinnah Women University (FJWU), Rawalpindi.

Preparation of Adsorbent: The *Aspergillus fumigatus* biomass was prepared in PD broth (potato dextrose broth) media. To prepare 100 ml of the PD broth 30ml of potato broth and 2 gm of glucose was added in conical flask and were filled up to 100ml of distilled water. Flask was tightly closed with a cotton plug and then aluminum foil and autoclaved at 121 C and 15 pascal for 20 minutes. Later on the flask was opened under laminar flow and fungus was inoculated into each flask. The flasks were agitated on a rotatory shaker for 3-4 days at 150 rpm and at 30ºC temperature. After 3-4 days thick bed of fungal biomass developed was further used for biosorption experiment.

Metal Biosorption Experiment: To investigate the biosorption capacity of the several *Aspergillus fumigatus* isolates towards the heavy metals with various initial heavy metal concentrations and optimal cultural conditions, were employed (table-1). Metal solutions of 200ppm, 400ppm, 600ppm and 800ppm were prepared with $CuSO_4$, $Pb(NO_3)_2$, $ZnCl_2$, $Cr(NO_3)_3$, $Ni(NO_3)_2$ and $CdCl_2$ for metals copper, lead, zinc, chromium, nickel and cadmium, respectively. The pH of metal solution is adjusted at 5.0 by using 1N HCl or 1N NaOH. Subsequent to metal solution preparation, 1 gm of fungal biomass was suspended in 100 ml of metal solution in 250ml conical flask. The flasks were agitated on a rotatory shaker at 150rpm and at 30ºC with contact time of 4 hrs. The initial pH and biosorption contact time was chosen based on previous studies reported[15-17].

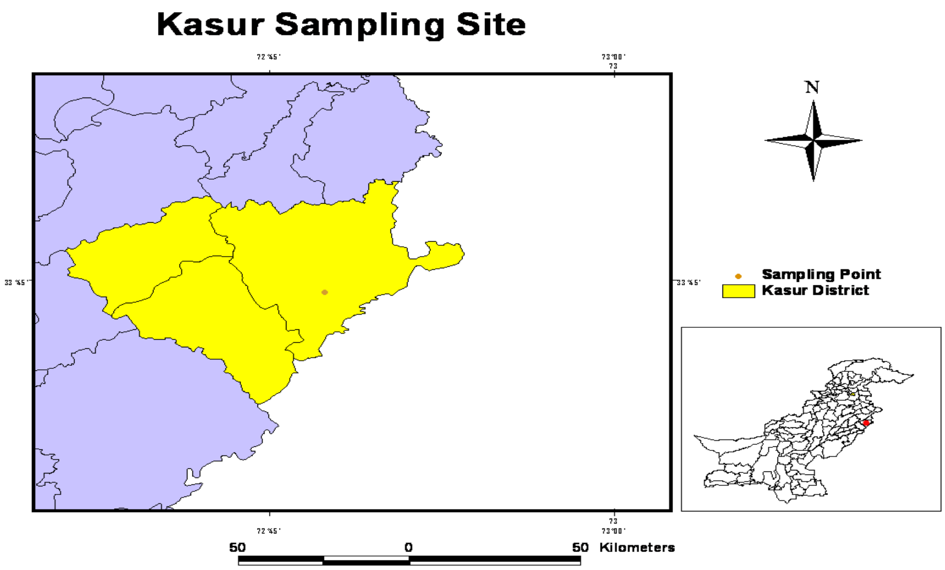

Figure-1
Study area and Sampling site located in Kasur district, Pakistan

Table-1
List of *Aspergillus fumigatus* isolates used for biosorption analysis collected from the polluted soil in Kasur district

Sr. No.	Fungus Name	Isolate Codes
1	*Aspergillus fumigatus*	K3
2	*Aspergillus fumigatus*	K4
3	*Aspergillus fumigatus*	K12
4	*Aspergillus fumigatus*	K18
5	*Aspergillus fumigatus*	K26
6	*Aspergillus fumigatus*	K27
7	*Aspergillus fumigatus*	K28
8	*Aspergillus fumigatus*	K29
9	*Aspergillus fumigatus*	K30

After each experiment employed different fungal isolate and metal concentrations, the mixture was filtered through Whatman filter paper no.1 and the filtrate obtained was examined by atomic absorption spectrophotometerfor metal concentration.In order to run the samples in atomic absorption spectrophotometer the concentration of the metal solution was reduced by dilution of metal solution after each experiment. The 1 ml of metal solutions of concentration 200ppm and 400pm were diluted up to 250ml in volumetric flask with distilled water and the metal solutions of concentration 600ppm and 800ppm were diluted up to 500ml in volumetric flask with distilled water. The samples were stored in test tubes and were analyzed further in atomic absorption spectrophotometer[18].

Biosorption Analysis of Data: The experiment was performed in three replications keeping the experimental conditions constant. The total quantity of metal ions biosorbed per gram of biomass for each sample (Q or q) was evaluated. Biosorption capacity was measured by employing following formula:

$$q = \left(\frac{C_i - C_f}{m} \right) V$$

Whereas ***q***=mg of metal ions uptake per gram biomass (mg/g), C_i=initial concentration of the metallic ions (mg/L); C_f=final concentration of metallic ions (mg/L); ***m***=dried mass of the biosorbent in the reaction mixture (g) and ***V***=volume of reaction mixture (ml).

Results and Discussion

The heavy metal ions are present in natural and industrial disposed wastewater. These metallic ions present on the surface and underground water resulted in soil contamination. Many conventional techniques have been practice to eliminate heavy metal ions including physical (membrane separation, ion exchange) and chemical (neutralization, precipitation) techniques[16]. However, these methods are only efficient to eradicate mass of heavy metal present at high or moderate concentration but ineffective at diluted or low concentration of metal ions[19].

Biosorption technique employing microbial biomass as biosorbent has been illustrated. In this process both alive and heat killed dead biomass of several filamentous fungi (*Mucor*spp., *Aspergillus*spp., *Penicillium* spp., *Rhizopus* spp.) have been employed[20].

In the present study highly tolerant filamentous fungal isolates of *Aspergillus fumigatus* were isolated from the polluted soil of Kasur and biosorption capacity was checked against heavy metals viz.; Pb, Cr, Cd, Ni, Cu and Zn. In the present study pure culture of 9 fungal isolates of *Aspergillus fumigatus* were collected and there biosorption capacity was evaluated against heavy metals at different concentrations at constant pH 5 and similar methodology was reported[21] in biosorption experiment. Biosorption is commonly refers to the technique of passive binding of radioactive elements or metallic ions by active and denatured biomass. These biological agents possess property of metal-sequestering and can be utilized to reduce metal ions concentration of in the aqueous solution even from ppm to ppb level.

The results of biosorption vary from specie to specie because the process is dependent on factors including: fungal species, biosorbent size, metal solution concentration, solution pH, shaking time and ionic composition. Fungi constitute a high proportion of the microbial biomass in soil. Being widespread in soil their large surface to volume ratio and high metabolic activity, fungi can contribute significantly to heavy metal dynamics in soil[22].

In the present investigation, the biosorption capacity using different fungal isolates are influenced by the concentration of metal ions. The peak value was obtained for metal lead on all the metal ion concentration. The fungal isolate K3 of *Aspergillus fumigatus* exhibited the maximum biosorption rate (76.07) at 800ppm as moving towards higher concentration against the metal lead (figure-2). The pattern of behavior of isolates K3 and K26 was almost linear compare to K4 against metal lead. Alternatively, isolates K3 and K4 showed maximum absorption (69.6) against copper following lead (figure-3). On observing the polynomial pattern, both isolates showed non-linear behavior. Figure-4 presents the biosorption capacity for metal chromium and it was evaluated that all the three isolates (K18, K29, K30) represented different pattern of absorption on atomic spectrophotometer. The absorption behavior for cadmium was characterized as non-linear (figure-5). On contrast to nickel and zinc metals, the least absorption values (25 and 20, respectively) at 800ppm were investigated by isolates K3, K12 and K18. However, K3 showed linear pattern compare to K12 and K18 (figure-6-7).

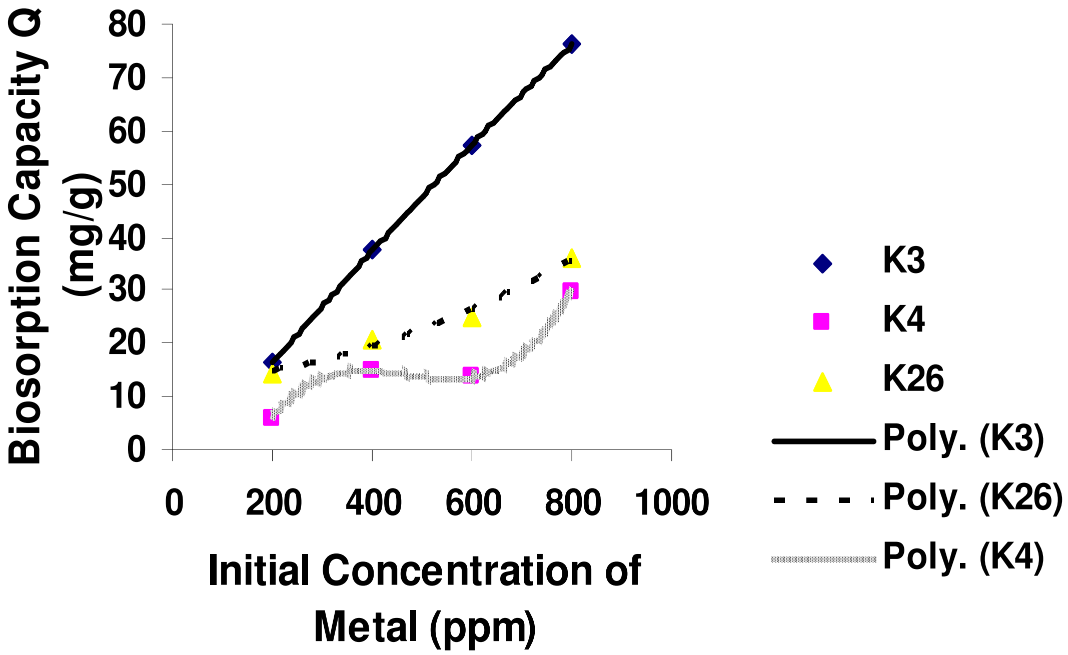

Figure-2
Effect of biosorption capacity on different concentrations of lead ions using K3, K4 and K26 isolates of *Aspergillus fumigatus*

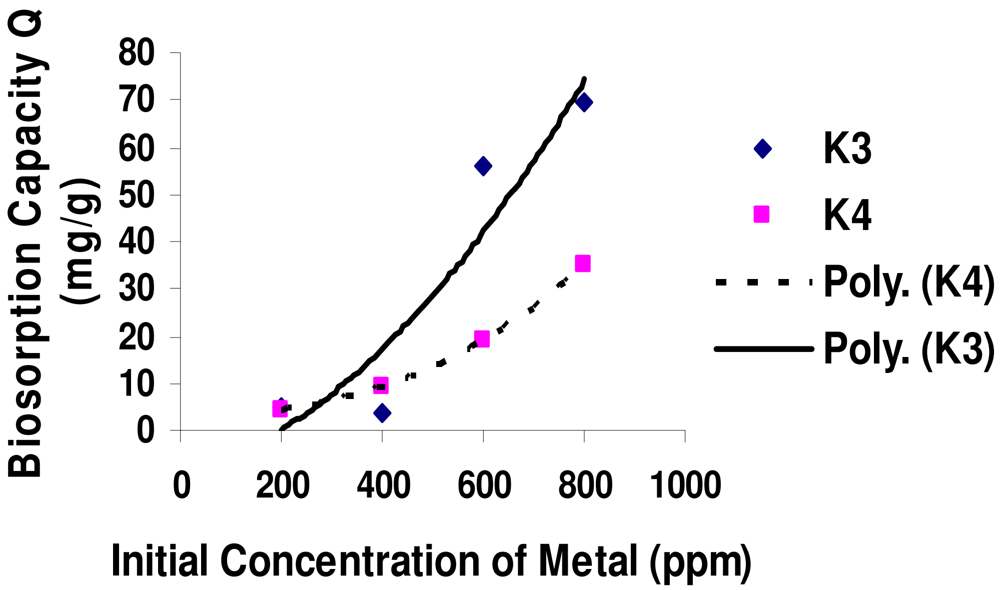

Figure-3
Effect of biosorption capacity on different concentration of copper ions using K3 and K4 isolates of *Aspergillus fumigatus*

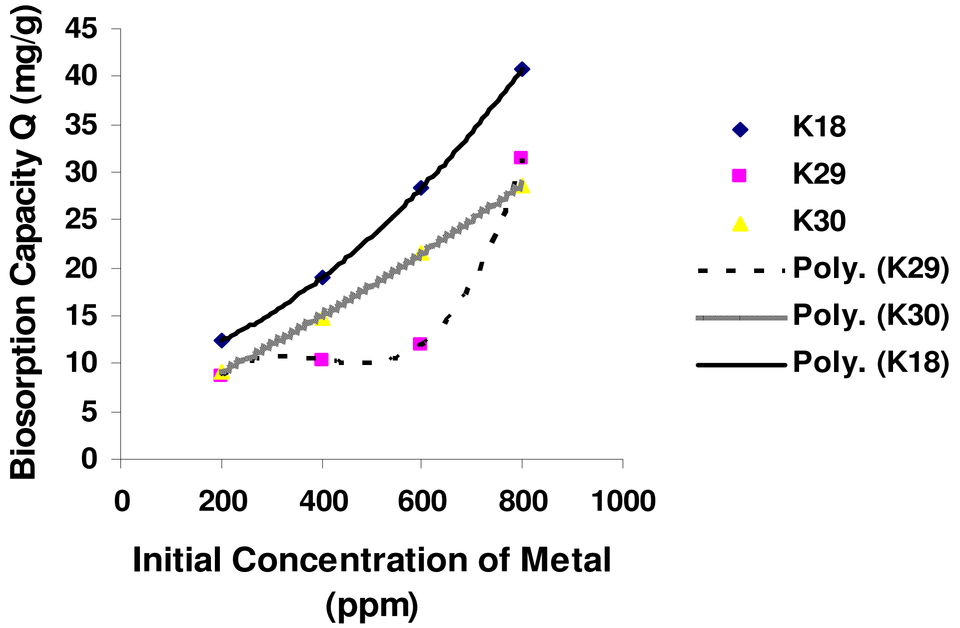

Figure-4
Effect of biosorption capacity on different concentrations of chromium ions using K18, K29 and K30 isolates of *Aspergillus fumigatus*

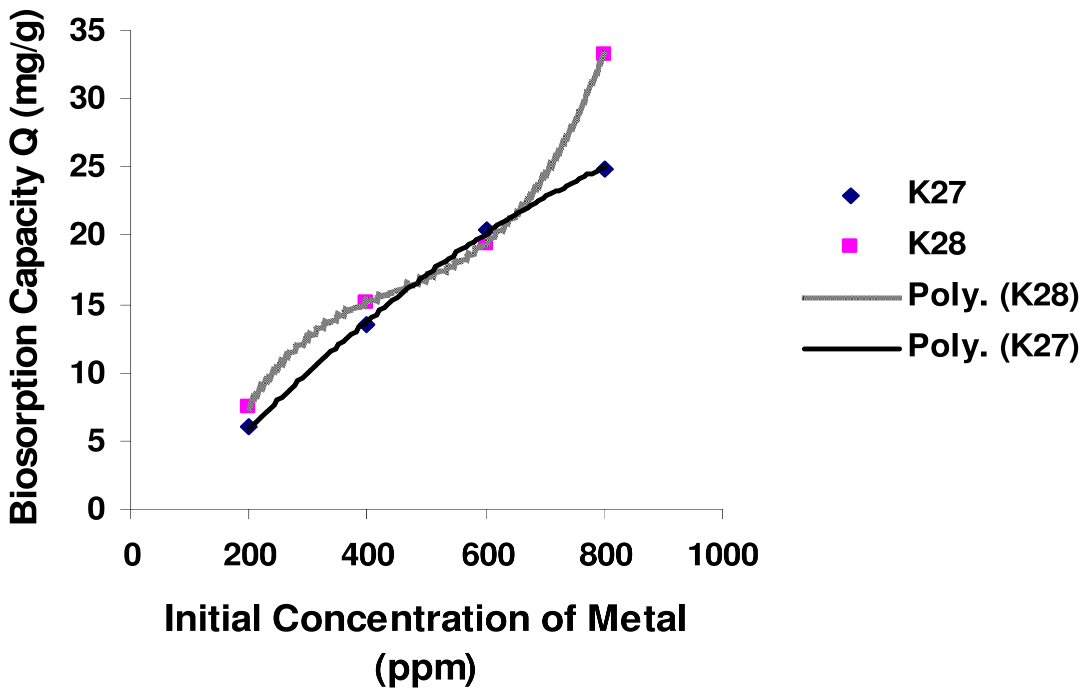

Figure-5
Effect of biosorption capacity on different concentrations of cadmium ions using K27 and K28 isolates of *Aspergillus fumigatus*

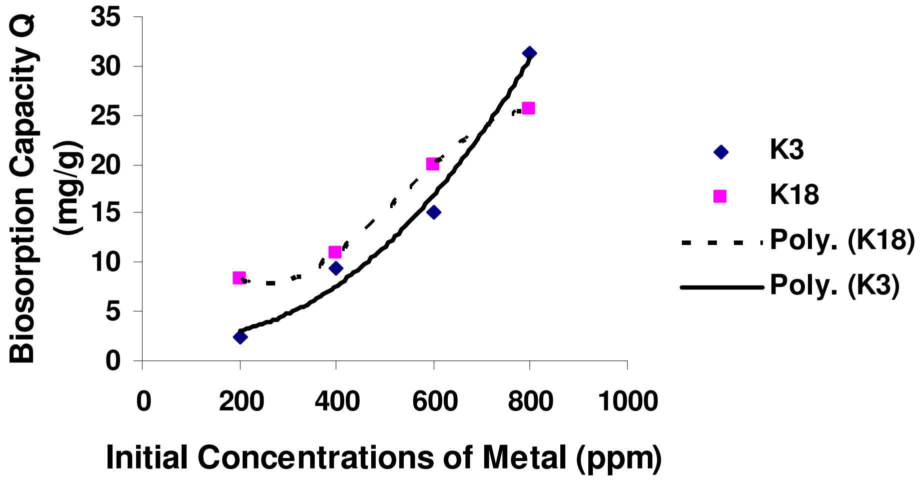

Figure-6
Effect of biosorption capacity on different concentrations of nickel ions using K3 and K18 isolates of *Aspergillus fumigatus*

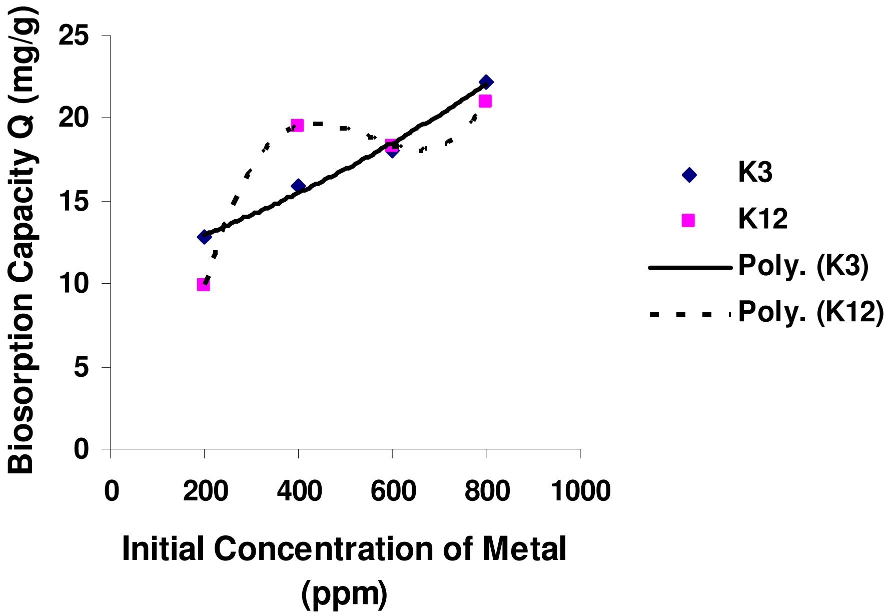

Figure-7
Effect of biosorption capacity on different concentrations of zinc ions using K3 and K12 isolates of *Aspergillus fumigatus*

The rest of conditions like pH, temperature, time and revolution speed were kept constant throughout the experiment. In one such study the heavy metal biosorption and tolerance of filamentous fungus from the soil polluted with metal was evaluated[21]. The results demonstrate that highest biosorption capacity of Cr was shown by 6mM concentration. The varying degree of biosorption capacity is mainly due to employing different isolates and change in the biomass. In another studied the biosorption of lead (Pb) by indigenous fungal isolates at various concentrations and peak value for Pb removal was observed on 1000 mg/L[23]. Similarly, according to another study reported the investigation of *Aspergillusniger* biosorption that is reflecting the similar pattern of biosorption[24]. Similar conducted work on biosorption of heavy metals by fungal dead biomass[25]. The finding depicted more sorption of lead at high metal solution concentration.

In our study, the pH was adjusted at 5.0. The uptake of heavy metals by *Aspergillus fumigatus* was found to be strongly influenced by the initial pH value of aqueous metal solution. It has been commonly convenient that pH of metal solution can intensely influence metal absorption intensity of biosorbents[26-29].

In the investigation, the biosorption time was adjusted at 4hrs. The total quantity of absorption metal ions was highest at the start of absorption and saturation points were completely reached at about 4 hours for all metals ions. The binding of the metal ions with functional group of fungal biomass is already reported. A wide range of equilibrium biosorption times are reported with various biosorbent systems.

Due to rapid global urbanization and ultimately industrialization the relationship between human health and environmental pollution has been seriously recognized. However, the most challenging aspect of environmental pollution in the modern civilization is to control the human activities[30-33]. Therefore, this has to be need of hour to properly manage public activities that are mainly the key factors in contributing the environmental pollution.

In the conclusion, the highest biosorption capacity was shown by K3 isolate against metal lead and copper with almost linear polynomial pattern followed by K18 against chromium. The ability of the filamentous fungi to biosorption and accumulate metals together with tremendous properties of fungal mycelia to offer an opportunity utilizing such candidates in selective adsorption of heavy metal from waste water and soil nearby industrial sectors.

Conclusion

The knowledge of present investigation will provide information about heavy metal biosorption by filamentous fungus. This high absorption capacity of fungus made them well suited for removal of heavy metal present in very low or diluted concentration from polluted water, bioleaching, bioremediation of polluted sites and effluent treatments. The information of present study will be helpful for further assessment and management of natural biosorbent (fungus) which could serve as an economically good source of treating industrial effluents contain toxic heavy metal ions.

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