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Original Article

A Biosorption study of Lead by Aspergillus Fumigatus

Anil Gill[1], Akash John[2], Tallat Anwar Faridi[3], Sofia Noor[4] and Neelam Iqbal[3]

[1] Gujranwala Institute of Medical and Emerging Sciences, Gujranwala, Pakistan
[2]University Institute of Radiological and Medical Imaging Sciences, The University of Lahore, Gujrat Campus, Gujrat, Pakistan
[3] University Institute of Public Health, The University of Lahore, Lahore, Pakistan
[4]Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Pakistan

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Correspondence Address:
Anil Gill
Gujranwala Institute of Medical and Emerging Sciences, Gujranwala, Pakistan

Abstract

Heavy metals liberated by various industrial and agricultural processes are the major pollutants in soil, agricultural, marine, and industrial and even treated waste waters. Lead is greatly used in many industrial applications such as a storage battery manufacturing, printing, fuels, photographic materials, pigments and explosive manufacturing **Objective:** To assess the ability of *Aspergillus fumigatus* for removal of metal contamination such as lead by biosorption **Methods:** The pure culture of the *Aspergillus fumigatus* was used for biosorption. The initial samples were cultured on the bread as the fungal spores were given suitable environmental conditions i.e., temperature, moisture, pH etc. For obtaining the pure culture of the *Aspergillus fumigatus* culture media was prepared. The spores collected were then allowed to grow on a specific culture media in a sterile Petri plates under aseptic conditions. Inoculation will be carried out by using 20 loops of fungal spores spread on the culture medium. After inoculation these plates were incubated at 28°C for few days and the colonies of the fungal strain becomes visible after 48 hours. The prepared agar is poured in the sterilized Petri plates were allowed to cool and solidify. As a result of this centrifugation, the spores and agar were separated. After 12 days of incubation, one fourth portion of the agar plate was cut and placed into the falcon tubes containing 10 ml distilled water **Results:** The results revealed that the biosorption recovery rate is maximum at 120 mg\g of dry mass i.e. 3.4%. The above estimation shows that the percentage of biosorption is directly proportional to the biomass concentration.

Introduction:

Release of heavy metals in the environment due to various industrial and agricultural activities is a major concern due to [1] the toxicity of these metals in humans and other life forms. Even at very low doses, several of them are exceedingly poisonous to a wide range of organisms (in the order of milligrams or micrograms per liter) [2]. Furthermore, these ions are non-degradable and consequently persistent, posing environmental and health risks even at very low concentrations (in the order of milligrams or micrograms per liter). Additionally, these ions are non-degradable and consequently persistent, posing environmental and health risks [3]. Heavy metal ions in the environment at concentrations beyond critical values are therefore unacceptable, and their removal from wastewater is of paramount importance [4, 5] . As a result of industrial activity and technological advancement, heavy metal emission into the environment has been steadily increasing. It poses a serious threat to the ecosystem, as well as human and soil health. [6] Heavy metal pollution has naturally become one of the most serious environmental problems as a result of their expanding application and unchangeable nature in recent years. As a result of industrial activity and technological growth, heavy metals such as copper (Cu) and lead (Pb) are continuously discharged [7] into the environment as a result industrial activities and technological development [8]. Lead is one of the most important heavy metals because, if deposited in high proportions, it poses a serious threat to people [9]. Petrol combustion accounts for almost 60% of total lead emissions worldwide. Lead contamination from automobile exhaust is the primary source of lead in soil and vegetation [10]. Battery manufacture, printing, pigments, fuels, photography materials, and the paint industry all use lead [11]. In other investigations, Pb levels in the air ranged from 33.3 to 338.7 g/m3, in soil from 0.62-21.2 mg/kg, and in water from below detectable levels to 170 g/L [11, 12]. Due to lead poisoning, the field personnel of the Lahore traffic police is said to be suffering from hemolytic anemia [13]. Chemical precipitation, ion exchange, electrochemical treatment, and membrane technologies have all been investigated in depth as traditional methods for eliminating metal ions from aqueous solutions. These methods, however, are unsuccessful and inefficient [14]. As a result, quick, cost-effective, and environmentally friendly solutions are required to develop [15].

Biosorption is a phrase that refers to the employment of microbes to detoxify and control pollutants in the environment. Cleaning up polluted places has recently gotten a lot of attention. [16]. This alternate method for removing heavy metals and radionuclides from aqueous solutions has been deemed the preferred strategy since it uses nonviable microbial biomass such as algae, fungus, and bacteria [17] . Biosorption, which is based on interactions between living and non-living microbes and metallic ions in the system, has a low operating cost and high effectiveness for eliminating low levels of heavy metal from wastewater [18]. The fungal cell wall is mostly composed of polysaccharides, proteins, and lipids, according to studies on the mechanism of heavy metal biosorption on fungus. There are numerous functional groups in these that are responsible for metal binding [19]. In the current study, the removal of lead (Pb) by fungal biomass i.e. *Aspergillus fumigatus* and the influence concentration of metal on biosorption of lead is explored.

Methods:

Micro-organism and Collection of samples

The pure culture of the *Aspergillus fumigatus* was used for biosorption. The initial samples were cultured on the bread as the fungal spores were given suitable environmental conditions i.e., temperature, moisture, pH etc.

Media preparation

Culture media was created to obtain a pure culture of Aspergillus fumigatus. Sabouraud

Dextrose Agar (SDA) is a Dextrose Agar modification. Pathogenic and commensal fungi and yeasts are cultured using SDA. The formula's high dextrose content and acidic pH allow for fungal selectivity. Suspended 65 g of the medium in one liter of purified water. Then boiled for one minute with regular stirring to completely dissolve the medium and autoclaved for 15 minutes at 121°C. Excessive heating of the medium will denature the agar in an acid pH, thus resulting in a medium which will be too soft.

Isolation of pure culture

The spores collected were then allowed to grow on a specific culture media in a sterile Petri plates under aseptic conditions. After that, an inoculating loop was heated to red hot, cooled, and utilized to transfer some of the spores into a sterile culture medium plate. As the spores were directly transferred to the culture media they were kept for few days and observed. The process was repeated until a pure culture is gained.

Inoculation and Incubation

Inoculation was carried out by using 20 loops of fungal spores spread on the culture medium. After inoculation these plates were incubated at 28°C for few days and the colonies of the fungal strain becomes visible after 48 hours.

Metal solution and Preparation of metal amended agar

A stock solution for lead ions (1000ppm) was prepared by dissolving an accurate quantity of $Pb_2(NO_3)_2$ in deionized water. To prepare metal amended agar, different concentration of metal solution according to the requirement was added to the distilled water and after heating it measured amount of agar was added to it. The prepared agar is poured in the sterilized Petri plates were allowed to cool and solidify.

Inoculation and Incubation on metal amended agar

Inoculation on the metal amended agar was done by the same method as done earlier. After inoculation these plates were incubated at 30°C for 10 days. The growth determined the biosorption rate of fungal strain for a particular metal used.

Centrifugation

After 12 days of incubation, one fourth portion of the agar plate was cut and placed into the falcon tubes containing 10 ml distilled water. These falcon tubes were then placed into the centrifuge machine. These 15 falcon tubes were then centrifuged at 1000rpm. As a result of this centrifugation, the spores and agar were separated.

Metal analysis and Digestion

Sulphuric acid and hydrogen peroxide were used to break down the fungal metal. The fungus was collected in digestive tubes. 1ml of H2O2 (35 percent analar reagent extremely pure) was poured down the side of the digestion tubes and turned after 2ml of conc. H2SO4 was added and incubated over night at room temperature. After the reaction was completed, the tubes were placed in the digesting block and heated to 350°C until fumes were created, then continued to heat for another 30 minutes before being taken from the block and cooled. 1 ml H_2O_2 was added, chilled, and the tubes were placed back into the digesting block for 20 minutes until fumes were formed. The steps above were repeated until the cooled substance was colorless. The extract was produced up of 50ml of distilled H_2O, filtered, and used for mineral element analysis.

Atomic absorption spectrometry

The essential principles of atomic absorption spectrometry were established in the second part of the nineteenth century by Robert Wilhelm Bunsen and Gustav Robert Kirchhoff, both professors at the University of Heidelberg in Germany. The presence of metals in liquid samples is determined using atomic absorption spectroscopy (AAS). Metals such as Fe, Cu, Al, Pb, Ca, Zn, Cd, and others are detected in the samples using AAS. Typical values are in the low milligrams per liter range.

Results:

The present study was conducted to find the biosorption rate of a heavy metal lead Pb^{2+}, by a

fungal Biomass of Aspergillus fumigatus. 15 plates of metal amended agar plates of different concentrations of Pb were prepared. The fungal spores were allowed to grow on the metal amended agar for 10 days. After 10 days the fungal growth was checked and the spores were separated by centrifugation. The spores were than digested and lead conc. was determined by Atomic absorption spectrometry. The results are recorded in tables and figures as given below in Table 1.

Sr.no	Lead (mg/L)	Lead (mg/g of dry mass)	Lead biosorbed (%)
1	3.5	120	3.4
2	7	122	1.74
3	10.5	125	1.19
4	14	127	0.9
5	17.5	129	0.74
6	21	156	0.74
7	24.5	203	0.83
8	28	233	0.83
9	31.5	241	0.76
10	35	127	0.36
11	38.5	123	0.32
12	42	120	0.28
13	45.5	117	0.26
14	49	102	0.21
15	52.5	93	0.18

Table1: Biosorption rate of lead at different concentrations

According to the above results by AAS shows that the biosorption recovery rate is maximum at 120 mg\g of dry mass i.e. 3.4%. The above estimation shows that the percentage of biosorption is directly proportional to the biomass conc. it also indicates that the recovery rate is inversely proportional to the metal concentration. As the metal conc. increases, it causes toxicity which results in the biomass destruction which decreases the recovery biosorption rate.

The tolerance of lead was measured for the lead concentrations ranging from 3-53mg/L and the extent of tolerance was compared. When the concentration of lead increased in the metal amended agar, the absorbance of the fungal culture decreased [20]it is due to the increased toxicity due to the increased level of the lead. As the lead conc. increases the ratio of the growth of *A. fumigatus* decreases and the microbial cells started to enter the death phase. Acc. to the results, the maximum lead absorption was given at 3.4mg/L i.e. 120mg/g after 10 days of incubation and the minimum absorption is recorded at 52.5mg/L i.e. 93mg/g of the dried biomass (Figure 1).

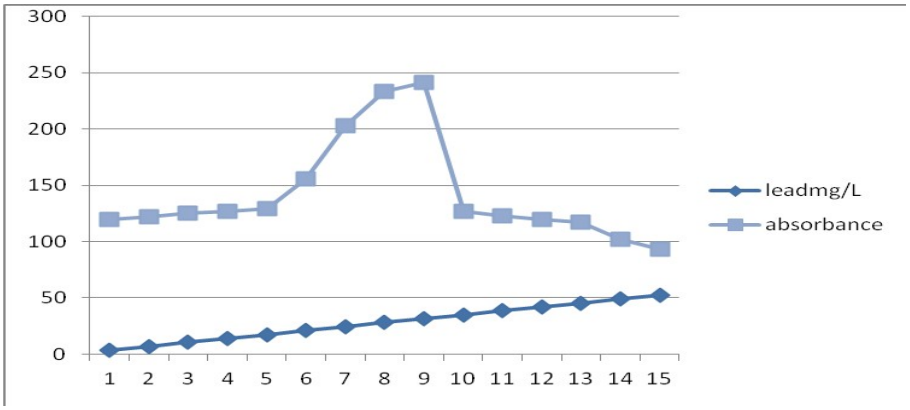

Figure 1: Concentration of lead (Pb) and its absorbance

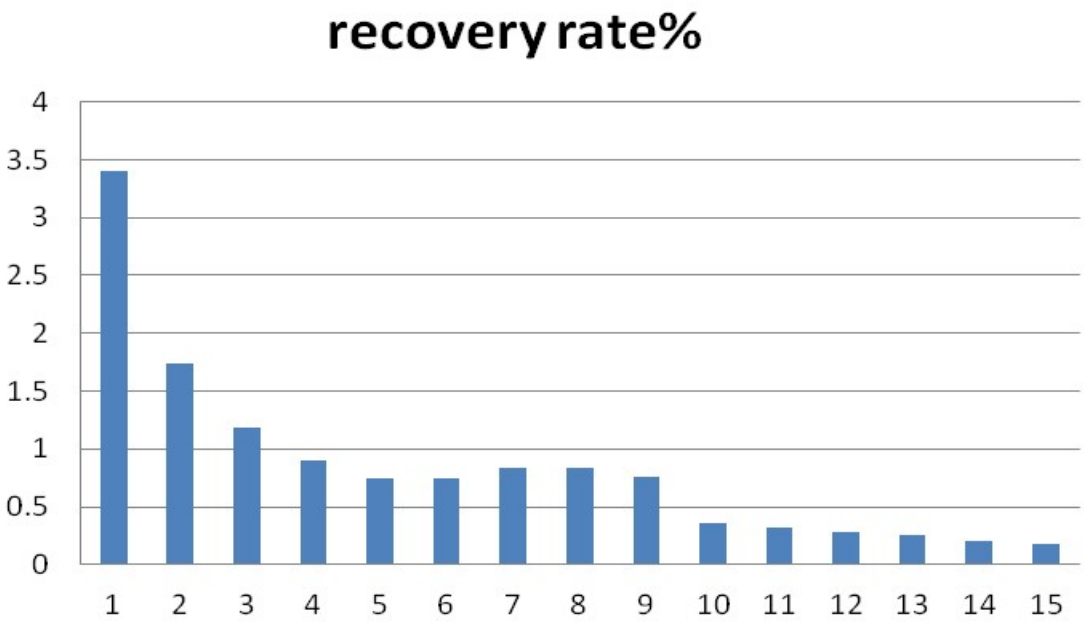

Figure 2: Recovery rate of lead (Pb) by Aspergillus fumigatus

Figure 2 shows the fluctuation of recovery rate of Lead (Pb). The highest recovery rate has been recorded at 120mg/g i.e. 3.4%. The values fluctuation occurs due to the toxic production of Lead. As the Pb accumulation increases by the Aspergillus fumigatus, Pb starts releasing toxins reaching at certain level which causes cell destruction resulting in the decrease of recovery rate.

Discussion:

Cell surface absorption permits interaction between harmful metal ions and functional groups such as carboxylate, hydroxyl, sulphate, phosphate, and amino groups present on the cell wall surface, whereas intracellular buildup of metal ions can occur by the cells metabolism using only live cells. Ion exchange, complexation, and physical adsorption are all used in these interactions [21].

Aspergillus fumigatus, a saprophyte that plays a crucial role in nitrogen and carbon recycling, was used in this study. It grows and develops naturally in the soil, where it lives and feeds on organic detritus. Aspergillus fumigatus has become the most common fungus in recent years, and it is a near-ubiquitous fungus with airborne conidia [22].

Metal contaminants are introduced into the environment in a variety of ways, each with potentially dangerous quantities. Accumulation of some elements, particularly hazardous heavy metals, can produce unintended changes in the biosphere with unpredictable repercussions. Microorganisms are the first recyclers in nature, turning hazardous chemical substances into innocuous products such as carbon dioxide and water. In present study, *A. fumigatus*, saprophytic fungi that played an important role in nitrogen and carbon is used as a microbial mass to absorb the heavy metal. In this present study *Aspergillus fumigatus* is selected for the biosoption of lead. Isolated fungal strain was allowed to grow on metal amended agar. The metal solution was prepared as 1g/l. According to different researches, some scientist has used lead as100mg/l [20]whereas 1000mg/l has also been used [23] 15 batches were run of different metal conc. ranging from 93-223 mg /l. All the tests were run at temperature 30°C) [23].

Conclusions:

In conclusion, it has been proved from the above results that bioisorption is the best process for removal of metal contamination. *Aspergillus fumigatus* has a great ability to absorb lead (Pb).

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