# BIOSORPTION POTENTIAL OF BACTERIA, FUNGI, ALGAE, AND PLANT EXTRACT FOR DETOXIFICATION OF HEAVY METALS IN INDUSTRIAL Effluents

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**Abstract**

In the era of industrialization, many industries produced heavy metal contaminants in large amounts, which is a big challenge for researchers to remove these contaminants from the environment (soil and water). We examined the metal biosorption potential of identified bacterial strains (*Pseudomonas aeruginosa* and *Staphylococcus aureus*), fungal strains (*Aspergillus Niger* and *Candida albicans*), *Parthenium Hysterophorus* plant extract, and *Spirogyra sp.* in marble industrial wastewater. Atomic absorption spectroscopy analyzed the heavy metals identification and quantification. The concentration of metals in wastewater was 8.568 mg/l Chromium, 5.053 mg/l Lead, 0.269 mg/l Cadmium and 0.411 mg/l Copper. The biosorbent showed a higher removal efficiency for Chromium, Lead, Cadmium, and Copper biosorption. We also carried out different parameters' effects on biosorption in the current study, i.e., temperature, contact time, and pH. The optimum pH was 5 for both bacterial strains, 7 for *Aspergillus Niger* and 9 for Candida albicans, 10 for *Parthenium Hysterophorus* plant extract, and 8.4 for *Spirogyra sp.*, showing the highest adsorption. The optimum temperature was 36°C for the selected bacterial and fungal strains and 40°C for plant extract. The optimal contact time was 21 days for all the biosorbents. Heavy metal is toxic when present in an increased amount in the environment. According to our study, a decrease in the heavy metal contaminants concentration is evidence that the toxicity of the heavy metals may also reduce. Heavy metals biosorption by *Parthenium Hysterophorus* plant and *Spirogyra sp.* is more desirable than bacteria and fungi because of their bioavailability, non-morbific, ease of cultivation, and eco-friendliness. The adsorption of each metal was rapid, which might be advantageous for large-scale remediation of polluted sites.

**Keywords:** Heavy metals, biosorption, bacterial and fungal strains, plant extract, *Spirogyra sp.*, pH, temperature, and contact time

## Introduction

One of the essential elements of life is water. Various natural and anthropogenic activities lead to surface water pollution, threatening human health [1]. Metals are one of the significant and dangerous pollutants of water waste. Even low amounts of heavy metals, such as Lead, nickel, Chromium, etc., as toxins can induce effects such as inhibition or modification of essential biological entities, including enzymes, DNA, and proteins [2]. Therefore, eliminating these harmful substances from effluents is necessary due to their adverse health consequences. Bioremediation is an eco-friendly and sensible approach for environmental decontaminants due to the minimization of chemical and biological waste, high efficiency, low cost and renaissance [3].

Environmental metal contamination emerges from natural or human factors, both direct or indirect, such as industrialization, anthropogenic sources, and urbanization, and poses risks to the environment and human health [4]. Natural events such as surface penetration of precipitation, volcanic phenomena, and forest fires also contribute to heavy metal contamination [5]. Mineral processing industries produce wastewater as the primary natural source of heavy metal pollution in water [6]. They pose an inherent danger to marine, animal, and human life, given their bio-accumulative, non-biodegradable, and toxic nature [7]. Non-essential metals such as Pb, Cr, As, Sb, Hg, and Cd are toxic in their chemically mixed forms and elemental structure [8].

Many industries, such as tanning, batteries, glassware, ceramics, electroplating, fertilizer, logging, art, and photography, discharge wastewater containing heavy metals such as silver, Chromium, Nickel, Cobalt, Manganese Iron, Copper, Mercury, Cadmium, Arsenic, and Lead. A variety of metals present in all wastewater are linked to an industry's operations. For example, the discharge of Chromium from tanneries in wastewater; Cadmium, Zinc, Copper, and Chromium are widely extracted from metal plating, refining, smelting, and burning of fossil fuels by electrical machinery and mining leads to the emission of mercury. Lead is produced from different mining and industrial sources. As a result, the heavy metal content in such wastewater exceeds the safe permissible limits and needs to be removed [9].

Biosorption is the measure of certain organic compounds for binding and concentrating chosen ions or other aqueous molecules [10]. Heavy metal biosorption has become a

significant subject in environmental research; it comprises only one critical component of the sorption method's intensity element. The technique of biosorption research is based on an interdisciplinary approach to biosorption which helps chemists, chemical engineers, and environmentalists to study and analyze the mechanism from multiple angles and perspectives [11]. In the present study, the potential of fungal and bacterial strains, plant extract, and algal biomass for remediation of Chromium, cadmium, lead, and copper was evaluated by characterizing the biosorption of these metals. Effect of temperature, pH, and contact time for heavy metals by these biosorbents were carried out.

## Methodology

### Materials

Wastewater samples were collected from the local marble industry at Buner KP, Pakistan. Two bacterial strains (Pseudomonas aeruginosa and Staphylococcus aureus), two fungal strains (Aspergillus Niger and Candida albicans), Algae (Spirogyra Sp.), and Parthenium Hysterophorus plant extract was used as a biosorbents. In addition, atomic Absorption Spectroscopy (AAS) was used to analyze the quantification of heavy metals in wastewater.

### Experiment

A biosorption experiment was conducted for heavy metals remediation in response to pH, temperature, and Contact time.

### Effect of pH

Five samples were prepared from 20ml wastewater containing 20ml nutrient broth media in 50ml falcon tubes. The pH of the solution was adjusted to 4, 5, 7, 9, and 10, respectively, using 0.1N NaOH and 0.1N HCl solution. 5ml culture of *Pseudomonas aeruginosa* was added to each sample. Each tube was incubated to grow bacteria in a solution in the shaker incubator for five days at 37°C at 80rpm to examine the biosorption potential. After five days, the samples were filtered, labeled, and subsequently analyzed [12]. The same experiment was followed for *Staphylococcus aureus, Aspergillus Niger,* and *Candida albicans*.

Five solutions of 30ml wastewater were taken in 50ml falcon tubes, and 10ml *Parthenium Hysterophorus* aqueous plant extract was added to each tube. The pH of the solution was adjusted to pH 4, 5, 7, 9, and 10, respectively, using 0.1N NaOH and 0.1N HCl solution. Each tube was incubated in a shaker incubator for five days at 37°C and 80rpm to examine the biosorption. After five days, the samples were filtered, labeled, and further analyzed.

Three samples of 100ml wastewater were taken in a 200ml glass beaker. In one of the wastewater samples, we added 0.5ml ammonium nitrate solution as a fertilizer, which reduced the pH of the sample from 7.8 to 4.8. The pH of the other two samples were adjusted to 7 and 8.4, respectively, using 0.1N NaOH solution. Subsequently, 1gm of fresh *Spirogyra sp.* was added to each beaker. All three samples were placed at room temperature for 24 hours to examine the biosorption potential. After 24 hours, the samples were filtered, labeled, and further analyzed.

**Effect of temperature**

Four samples were prepared from 20ml wastewater in a 50ml falcon tube containing 20ml potato dextrose media. 5ml of the bacterial strain of *Pseudomonas aeruginosa* was added to each sample. The pH of the sample was adjusted at 7 using 0.1N NaOH and 0.1N HCl solution through a pH meter. Each tube was incubated to grow bacteria in a solution in the shaker incubator for five days at different temperatures, i.e., 28°C, 32°C, 36°C, and 40°C, at 80rpm to examine the biosorption potential. After five days, the samples were filtered through filter paper and labeled for further analysis [13]. The same experiment was followed for *Staphylococcus aureus, Aspergillus Niger,* and *Candida albicans*.

Four samples were prepared from 30ml wastewater in a 50ml falcon tube. 10ml *Parthenium Hysterophorus* plant extract was added to each tube. The PH of the sample was adjusted at 7 using NaOH (0.1N) and HCl (0.1N) solution through a pH meter. Each tube was incubated in the orbital incubator for five days at different temperatures, i.e., 30°C, 35°C, 40°C, and 45°C at 80rpm, to examine the biosorption potential. After five days, the samples were filtered through filter paper and labeled for further analysis.

**Effect of Contact time**

Three samples were prepared from wastewater in a 50ml falcon tube containing 20ml Nutrient broth media. The pH of the sample was adjusted at 7 using 0.1N 0f NaOH and 0.1N of HCl through a pH meter. 5ml culture of *Pseudomonas aeruginosa* was added to each solution. Each tube was incubated to grow bacteria in a solution in the shaker incubator at 37°C for different contact times, i.e., 7 days, 14 days, and 21 days at 80rpm. After a specific contact time, the samples were filtered, labeled, and further analyzed [14]. The same experiment was followed for *Staphylococcus aureus, Aspergillus Niger,* and *Candida albicans*.

Three samples were prepared from wastewater in a 50ml falcon tube. 10ml *Parthenium Hysterophorus* plant extract was added to each tube. The pH of the sample was adjusted at 7 using 0.1N 0f NaOH and 0.1N of HCl through a pH meter. Each tube was incubated to grow bacteria in a solution in the shaker incubator at 37°C for different contact times, i.e., 7 days, 14 days, and 21 days at 80rpm. After a specific contact time, the samples were filtered, labeled, and further analyzed.

Two samples were prepared from 100ml wastewater in a 200ml glass beaker. The pH was adjusted to 8.4 using a pH meter by adding 0.1N NaOH solution. Then 1gm of fresh *Spirogyra sp.* algae was added to each beaker. The samples were placed at room temperature for 24 and 72 hours to examine the biosorption potential. After a specific contact time, the samples were filtered, labeled, and further analyzed

The biosorption percentage of metal was calculated with the help of the following formula.

$$Adsorption\ \% = \frac{C_0 - C_i}{C_i} \times 100$$

Where $C_0$ is the initial concentration of metals and Ci is the final concentration of metals [15].

## Results and Discussion

### Effect of pH

The adsorption process is enormously affected by pH, which can influence the surface charge of the adsorbent, the level of ionization, and the speciation of the adsorbate. The impacts of pH on Cr, Pb, Cd, and Cu adsorption on bacterial strains (Pseudomonas aeruginosa and Staphylococcus aureus), fungal strains (Aspergillus Niger and Candida albicans), Spirogyra Sp. and Parthenium Hysterophorus Plant extract were analyzed. The adsorption potential of Pseudomonas aeruginosa, Staphylococcus aureus, and Aspergillus Niger increased remarkably when the pH increased from pH 4 to pH 7 and then showed a decrease of adsorption in alkaline pH, while for *Candida albicans* the adsorption increased by increasing the pH up to pH 9 and then decreased at pH 10 which are shown in **Figure 1.**

The optimum pH for biosorption on Spirogyra Sp. was 8.4, which showed the maximum removal efficiency for Chromium, Lead, Cadmium, and Copper. On the other hand, the removal efficiency at neutral pH 7 was less than optimal, while at pH 4.8, the removal efficiency was much less than pH 7 and optimal. On Parthenium Hysterophorus plant extract showed maximum removal efficiency at optimal pH 10; the adsorption efficiency increased

by increasing the pH and reached the optimum, as shown in **Figure 2.** However, the effective adsorption of H+ ions dropped as pH increased, and the adsorbent surface remained more negatively charged. As a result, positively charged metal ions are easily adsorbed onto the adsorbent's negatively charged sites [16].

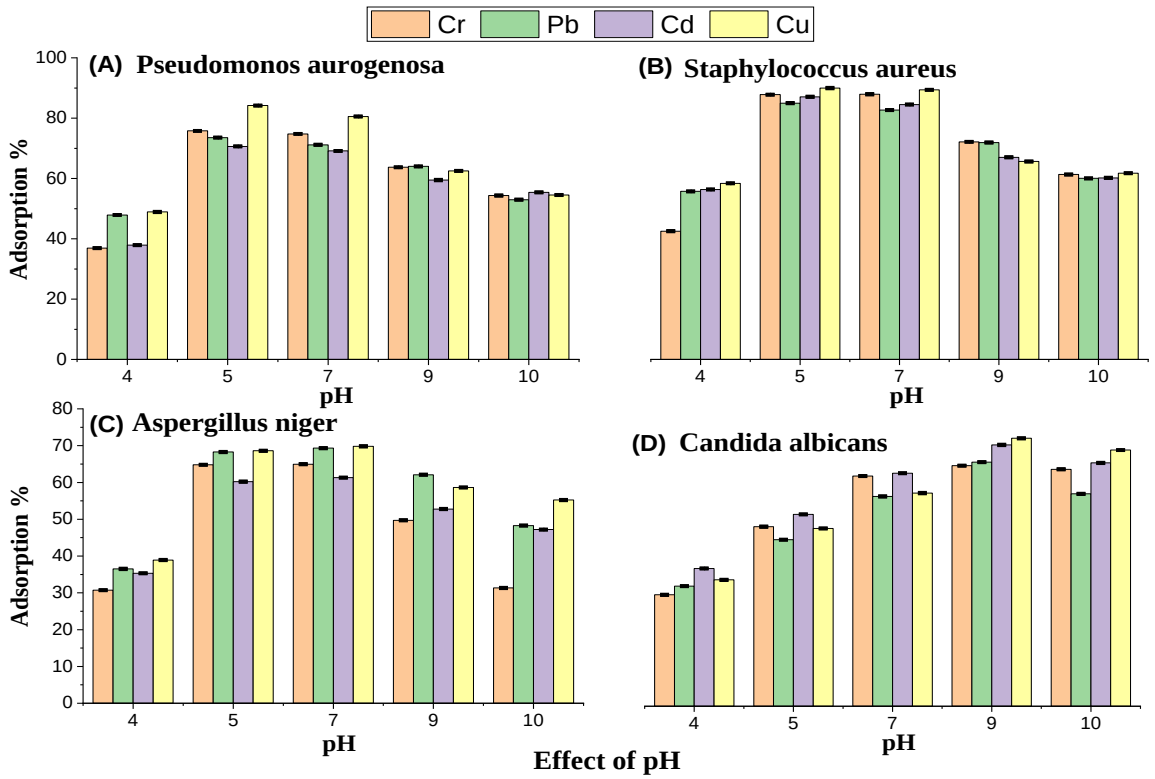


**Figure 1:** In the effect of pH (A) is the adsorption on Pseudomonas aeruginosa, (B) is the adsorption on Staphylococcus aureus, (C) is the adsorption on Aspergillus Niger, and (D) is the adsorption on Candida albicans

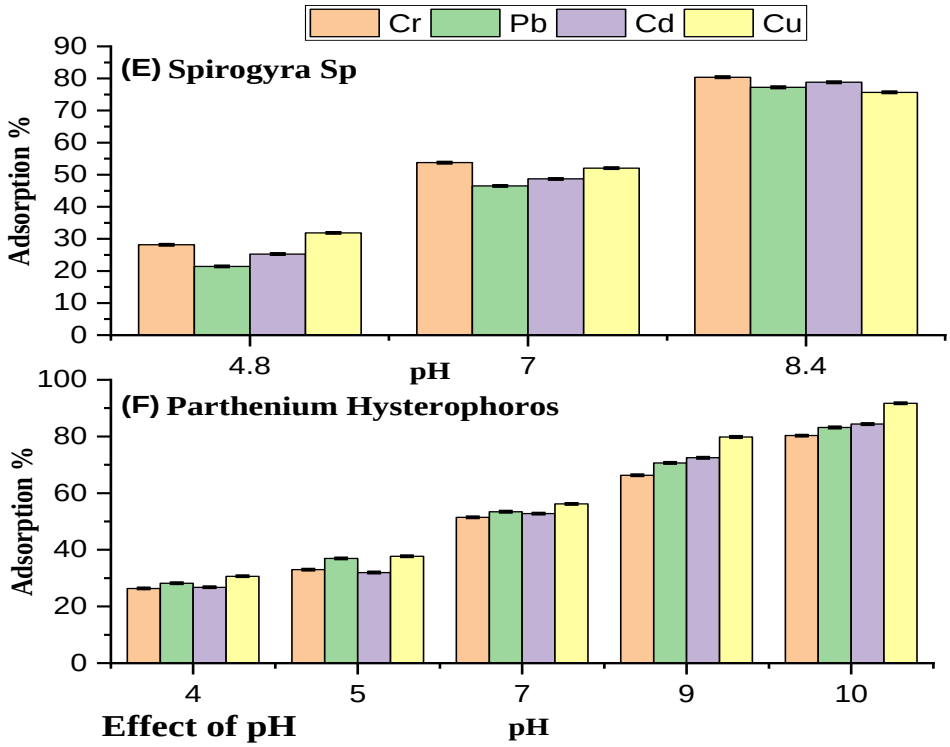


**Figure 2:** In the Effect of pH (E) is the adsorption on Spirogyra Sp. And (F) is the adsorption on Parthenium Hysterophorus plant extract

**Effect of Temperature**

Effect of temperature for the biosorption of Chromium, Lead, Cadmium and Copper on bacterial strains (Pseudomonas aeruginosa and Staphylococcus aureus), fungal strains (Aspergillus Niger and Candida albicans) at the temperature of 28°C, 32°C, 36°C, and 40°C and Parthenium Hysterophorus plant extract at the temperature of 30°C, 35°C, 40°C, and 45°C were investigated. The optimum temperature for (A, B, C, and D) in **Fig.3** was 36, which showed the maximum removal efficiency of Cr. The removal rate of Pb, Cd, and Cu increased by increasing the temperature and then reached the optimum. On the other hand, the removal rate showed a decrease when the temperature increased to 40°C, as shown in **Figure 3.**

By studying the effect of temperature on Parthenium Hysterophorus plant extract, the optimum temperature was observed at 40°C, which showed the maximum removal efficiency. By increasing the temperature, the removal rate increased and reached the optimum and then showed a slight decrease when the temperature rose to 45°C, as shown in **Figure 4.** The higher adsorption rate with increasing temperature might be due to higher affinity of the binding sites for metal cations or increased availability of the binding sites on the relevant cell surface. When the temperature increased over 40°C, it decreased the removal

rate, which might be due to the loss of binding sites available on the cell surface for metal removal. [17].

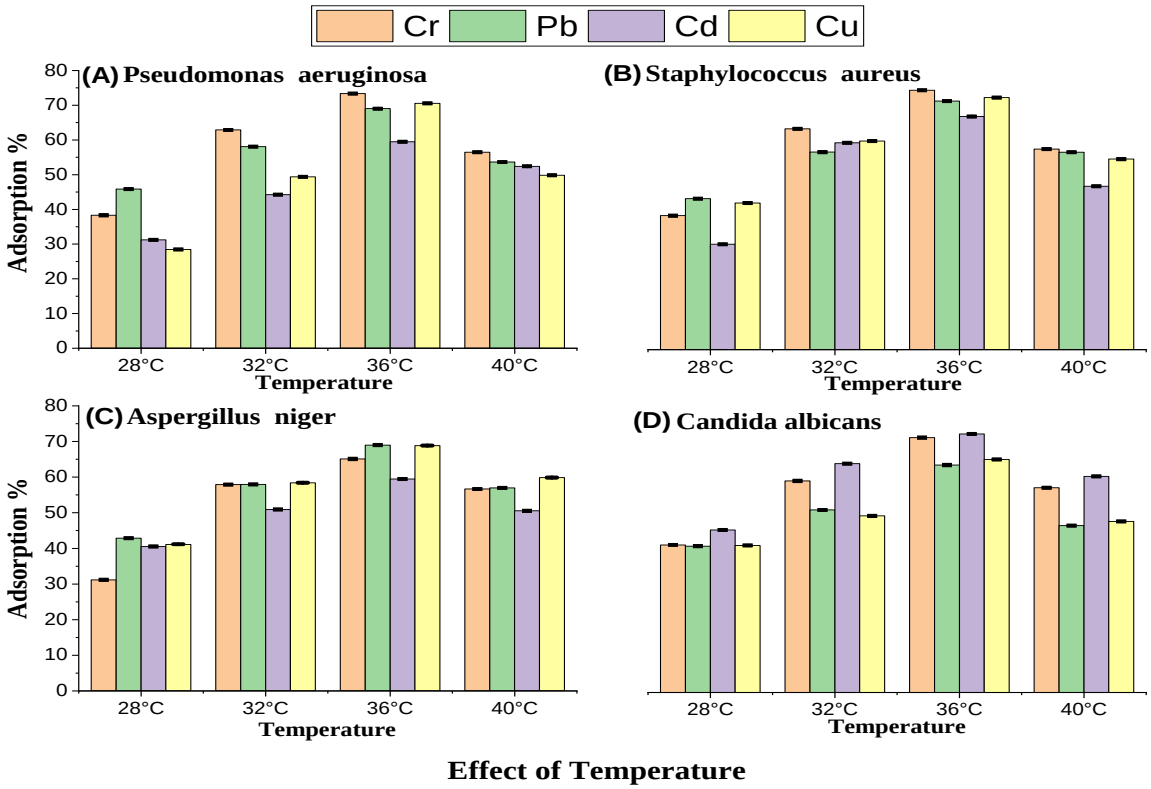


**Figure 3:** In the effect of temperature (A) is the adsorption on Pseudomonas aeruginosa, (B) is the adsorption on Staphylococcus aureus, (C) is the adsorption on Aspergillus Niger, and (D) is the adsorption on Candida albicans

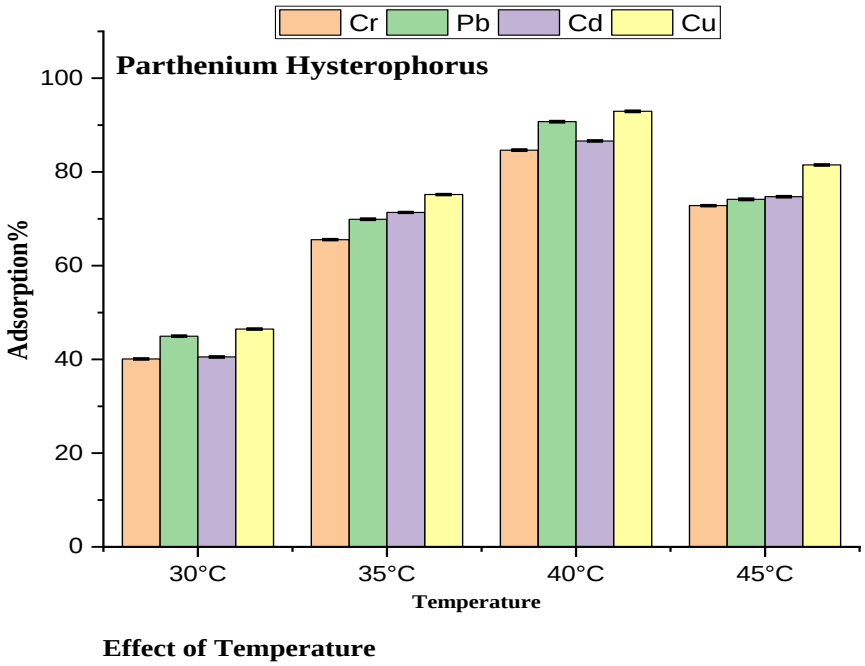


**Figure 4:** In the Effect of temperature (E) is the adsorption on Spirogyra Sp. And (F) is the adsorption on Parthenium Hysterophorus plant extract

### Effect of Contact time

The effect of contact is one of the essential parameters for the biosorption of heavy metals. In our study, biosorption of Cr, Pb, Cd, and Cu on bacterial strains (Pseudomonas aeruginosa and Staphylococcus aureus), fungal strains (Aspergillus Niger and Candida albicans), Spirogyra Sp. and Parthenium Hysterophorus plant extract were investigated. In the first seven days of the experiment, the adsorption rate was rapidly recorded; it might be because of the high availability of metal binding sites on the surface of adsorbents. On the other hand, the second and third-week results showed a slight increase compared to the first week of the experiment, as shown in **Figure 5.** Similarly, the contact time for Spirogyra Sp. was 48hrs and 72 hrs. The maximum adsorption rate was recorded at the contact time of 72 hours, resulting in approximately 90% of metal adsorption shown in **Figure 6.** This is a direct result of the accessibility of increasingly more adsorption sites for the complexation of metal ions [18].

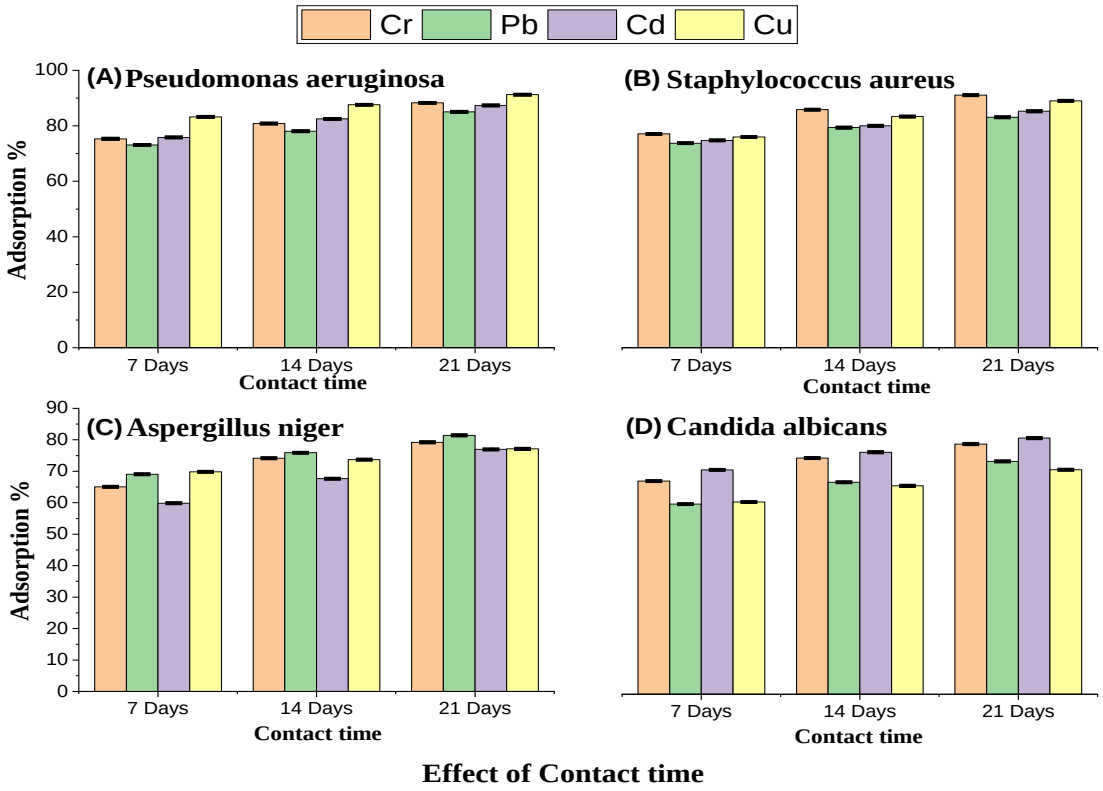


**Figure 5:** In the effect of contact time (A) is the adsorption on Pseudomonas aeruginosa, (B) is the adsorption on Staphylococcus aureus, (C) is the adsorption on Aspergillus Niger, and (D) is the adsorption on Candida albicans

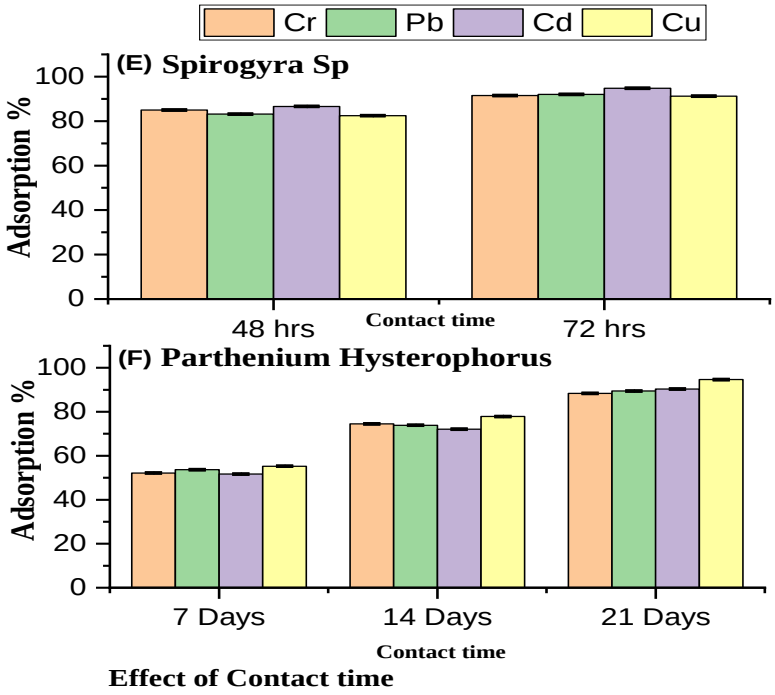


**Figure 6:** In the Effect of contact time (E) is the adsorption on Spirogyra Sp. And (F) is the adsorption on Parthenium Hysterophorus plant extract

## Conclusion

The present research aimed to find the biosorption potential of bacteria, fungi, algae, and plant extract to remove Chromium, Cadmium, Copper, and Lead contaminants from industrial effluent. Experiments were performed taking into consideration different parameters, such as pH, temperature, and contact time, to assess their role in the biosorption of heavy metals. The results showed the highest biosorption potential at optimum pH 5 for bacterial strains, pH 7 and 9 for fungal strains, pH 8.4 for algal biomass, and pH 10 for plant extract. The optimum temperature was 36°C for bacterial and fungal strains and 40°C for plant extract, while algae showed biosorption potential at room temperature. In addition, all the biosorbents showed a remarkable biosorption potential in 21 days of contact time. Bacterial and fungal strains have limited applications for use on a large scale due to their infectious nature. Therefore, *Parthenium Hysterophorus* plant extract and *Spirogyra sp* is the best choice having remarkable applications and can be employed in the future for heavy metals removal from wastewater.

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