ASSESSMENT OF HEAVY METALS UPTAKE BY SOME SELECTIVE BACTERIAL STRAINS ISOLATED FROM HAZARIBAGH TANNERY EFFLUENT IN DHAKA CITY, BANGLADESH: BIOSORPTION VIEWPOINT

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ABSTRACT

Biosorption can be defined as a Physiochemical process that cleanses heavy metal-contaminated water and aids in environmental remediation. The present exploration was carried out to examine the metal biosorbent potentiality of five bacterial strains.Biosorption capacity was assessed using Atomic Absorption Spectrophotometer where *Bacillus thuringiensis* showed uptake of Pb 0.56mg/g that was around 34.74%, Cd 0.38mg/g (20.0%), Ni 0.30 mg/g (15.8%), Cr 0.76mg/g (39.58%), Cu 0.14mg/g (7.61%) and As 0.28mg/g (14.43%) respectively. *Micrococcus sp.* showed reduction of Pb 0.62mg/g (39.89%), Cd 0.40mg/g (21.10%), Ni 0.22mg/g (11.6%), Cr 0.46mg/g (23.96%), Cu 0.56mg/g (30.43%) and As 0.03mg/g (1.75%) respectively whereas *Pseudomonas sp.* exhibited reduction of As 0.56mg/g and 0.20mg/g (16% and 10%), Cu 0.26mg/g and 0.46mg/g (14% and 25%), Cr 0.74mg/g and0.56mg/g (39% and 29%), Ni 0.12mg/g and 0.38mg/g (6% and 20%), Pb 0.26mg/g and 0.68mg/g (19% and 41%) and Cd 0.46mg/g and 0.64mg/g (24% and 34%) respectively. The result displayed that the highest degradation of Pb and Cr were shown by *Pseudomonas aeruginosa* (41±0.01%) and *Bacillus thuringiensis* (39.58±0.05%) and lowest degradation of As was shown by *Micrococcus sp.* that was around 1.75±0.001%. By contrast, degradation of Pb in moderate condition was witnessed by *Bacillus cereus* (19.89±0.01%) and Ni by *Bacillus thuringiensis* (15.8±0.05%) respectively. Heavy metal's biosorption is a quick process that might be an advantageous side for the removal of vast range of pollutants.

Keywords: Biosorption, Heavy metals, *Bacillus thuringiensis, Micrococcus sp. Pseudomonas sp.* Degradation

INTRODUCTION

Leather is one of the popular industries of Bangladesh which plays a pivotal role in the country's economy, as well as having a good international reputation. In the fiscal year 2016–2017, the leather sector contributed $1.39 billion to Bangladesh's economy, accounting for 4% of GDP (**Hoppe *et al*., 2009**). Bangladesh has about 113 tanneries that produce about one hundred and eighty million square feet of skins and hides annually. Notably, over 2500 smaller footwear manufacturers are engaged in making high-quality footwear from about 30 modern shoe manufacturing plants. Approximately 5058000 people perform their job in this sector. Most of the tanneries generate 20000m^3 tannery effluent and 232 tonnes of solid waste every day due to the lack of proper effluent plants. Though tannery has a potential value but liquid and solid wastes are potential contaminants (**Paul, 2013**).

Industrial effluent, which is heavily loaded with heavy metals, brings about health damage to animals, aquatic life, plants, and humans putting stress on the fauna as well as flora (**Robin *et al*., 2012**). **Li *et al.*, (2010)** reported the response of food crops to salinity stress in affecting the extent of heavy metal adulteration and changing their physiological and biochemical properties. Tannery effluent gets highly polluted because of the existence of conductivity, imbalance suspended solids, nitrogen, chromium, copper, cadmium, manganese, sulfate and sulfide, and chemical oxygen demand (COD) and biological oxygen demand (BOD) biological oxygen demand (BOD), and chemical oxygen demand (COD) (**Zahid *et al*., 2006; Mondal *et al*., 2005**). Tannery effluents discharged into aquatic systems and toxic metals associated; possess different environmental and public health concerns. Approximately 2 million people those are residing adjacent to the Buriganga River and Hazaribagh are somehow facing trouble by the waste of tannery (**Arias-Barreiro *et al*., 2010**).

Industrial wastewater, which is the source of various genres of organic and inorganic contaminants, is thrown away in receiving water compartments. These heavy metals, which are biologically non-biodegradable, play role in polluting the environment especially in high concentrations as well as their persistency in wastewater treatment, and their toxicity has become a global concern (**Jern, 2006**). For this reason, it is necessary to find out an eco-friendly solution to make the environment clean from metal contamination and therefore to improve the health progressively of the polluting environment (**Sekhar *et al*., 2004**). Therefore, the study focused on the Hazaribagh tannery that dropped away untreated tannery effluents including bio-toxic substances that cause serious health as well environmental issues in our community (**Igiri *et al*., 2018; Gain *et al*., 2002**). Hazaribagh is the principal leather processing zone which is located in the southern

part of Dhaka city. The random and mismanaged proliferation of Bangladesh's leather manufacturing company has had a detrimental impact on the surrounding environment, particularly land and water, posing a public health risk (**Alam *et al*., 2019**).

The tannery wastewaters containing high organic substances turn into a dark brown that differs according to the high presence of chemicals (**Kongjao *et al*., 2008**). The metal ion is concentrated through microorganisms by binding process (**Joutey et al., 2015**). The binding capacity of metal ions with protein and the prevention of DNA replication and the following cell division cause toxicity (**Kar *et al*., 1992**). Toxic metal pollutants of concern include arsenic, silver, chromium, selenium, nickel, zinc, uranium, cadmium, lead, gold, and mercury (**Ahalya *et al*., 2003**). Heavy metals enter into the food chain and ultimately in the human body, and lead to cause various life-threatening diseases and disorders (**Lee *et al*., 2007**). Various microorganisms have been originated to improve the process of detoxification to combat the negative impact of inorganic metals (**Igiri *et al*., 2018**). The harmful effects on humans and animals are the result of Heavy metal accumulation that affects the biota of the riverine ecosystem and microbiological balance of soils that reduce fertility (**Malik and Maurya, 2014; Barbieri, 2016**). Metal poisoning is responsible for causing disease symptoms like dementia in adults, mental disturbance in children, liver and renal diseases, and nervous system disorders (**Flora *et al*., 2008; Jan *et al*., 2011**).

Different conventional physicochemical methods used in remediation offer disadvantages like uneconomical, high reagent and energy requirement, and production of a huge amount of chemical waste, delayed metal precipitation, and unfinished removal(**Ahalya, 2003; Ayangbenro and Babalola, 2017; Aziz *et al.,* 2015**). Bioremediation through the use of microbes has received considerable interest and great attention over the years because it is a more cost-effective and eco-friendly method and efficient alternative approach to reduce or eliminate toxic heavy metals to safeguard the ecosystem compared with convention methods (**Igiri *et al*., 2018; Aryal *et al*., 2015**). Recently, **Gadd (2000)** stated the important application of microorganisms at high priority in the recovery of heavy metals from industrial effluents. **Volesky and Holan (1995)** decipher the fundamental mechanism of the biosorption process which is composed of three steps such as cell surface binding, extracellular participation, and intracellular bioaccumulation. Biotechnology possesses a significant advantage for the removal of heavy metals by entrapping metal ions, mainly through the process of biosorption using the capability of diverse bacteria and other microorganisms (**Lesmana *et al*., 2009**). The biosorption methods have been more popular than the conventional methods due to the affordability of biosorbent and it provides various benefits of metal recuperation as well as the regeneration of biomass (**Volesky, 2007**). In this

regard, the current study has investigated the potentiality of bacterial biomass as a biosorbent for the removal of heavy metals from environmental pollutants.

MATERIALS AND METHODS

The experiments were carried out in Soil and Environment Laboratory, Biological Research Division, Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka-1205, Bangladesh.

Microbial strains

In the current study, five microbial species were employed: *Bacillus thuringiensis*(Bt), *Bacillus cereus*(Bc), *Pseudomonas monteilii*(Pm), *Pseudomonas aeruginosa*(Pa), and *Micrococcus luteus*(Ml). All of the five microbes were previously isolated from tannery effluent at Soil and Environment Laboratory, Biological Research Division, Bangladesh Council of Scientific and Industrial Research.

Biomass preparation

Bacterial strains were maintained on casamino acid media (CAA), which is made up of 5 g/L casamino acids (Oxoid); K$_2$HPO$_4$, and 0.25 g/L MgSO$_4$.The optimum growth was determined by adjusting pH 5.5 (**Hussein *et al*., 2004**). The specific weight of each bacterial cell was harvested by performing a centrifugation process at room temperature for 15 minutes, which was afterward washed two times using then washed twice with autoclaved double distilled water. In deionized water, the cells were ultimately suspended to obtain a final concentration of 5 g/L.

Bacterial growth rate determination

The cell suspension's optical density was measured at the wavelength of 620 nm at different time intervals (0, 6, 12, 24, 30, 36, 48, and 60 hours) to assess the bacterial growth rate using a spectrophotometer.

Metal Solutions

Different concentration of metals was made by dissolving Cadmium Nitrate, Chromium (III) Nitrate, Nickel Nitrate, Cupric Sulphate, Lead (II) Nitrate, and Sodium Meta Arsenatesalts in deionized water to get a final concentration of100µg/mL. All the glassware was washed by using 0.1M HCl, so that metal elements cannot bind with them.

Metal biosorption by different isolates

Heavy metal biosorption tests were performed in an Erlenmeyer flask having 100 ml of each sample with10±1.0 mg of cells. The procedure of the current experiment is briefly shown in figure 01.
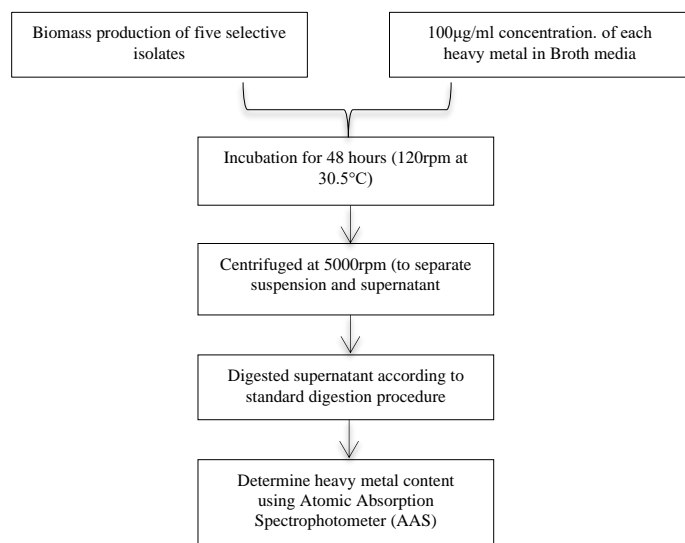

Figure 1 Flowchart showing the biosorption process for the removal of heavy metal

Cells, as well as waste, were kept under incubation for 48 hours without any interruption in agitation which was maintained at 120 rpm, at 30.5ºC to reach equilibrium. Cells were obtained via culture and procured from the same flask at the same growth phase in all assays. After 48 hours, cells were centrifuged at 5000 rpm to separate suspension and supernatant from the medium. Supernatants were digested according to standard digestion procedure and Atomic Absorption Spectrophotometer (AAS) was used to determine residual heavy metal concentration. To ensure proper bacterial growth in the batch culture, optimum temperature and pH were maintained accurately (**Hietala and Roane, 2009**).

Measurement of metal uptake

In the biosorption of the tested metals by the diverse bacterial strains, most of the metal ions were cloistered very fast from solutions within the first 15 minutes and almost no increase in the level of bounds metals have occurred after this time interval. The differentiation of the sorption execution of the different biosorbent was achieved under the same environmental conditions (i. e. pH, temperature and agitating speed, etc).
The formula used for the calculation of the amount of heavy metal bound is shown below.

$$Q = \frac{V(L) \; x \; (Ci - Cf)(\frac{mg}{L})}{S \; (g)}$$

Where Q is equal to mg of metal uptake of biomass per gram, S represents the mass of added cells on a dry basis (g), Ci symbolizes the initial conc., of metal in the solution (mg/l), Cf denotes final metal conc., in the solution (mg/l), and V represents the metal solution volume in ml.

Statistical Analyses

The data were analyzed statistically using a one-way analysis of variance (ANOVA) with a p=0.05 significance level. The data was processed into the Microsoft Excel 2010 software, which was used to compute the means and standard deviation.

RESULTS

Recent research activities have suggested microorganisms could be a good choice to rectify the contamination of metals by removing metals from contaminated waste materials, Recent studies have indicated that microbes might be used to rectify metal contamination by removing metals from contaminated waste, solubilizing metals or sediments, and sequestering metals in soils to assist in their extraction. The growth pattern of five different isolates was observed in nutrient broth media supplemented with 100ppm of total 06 individual heavy metals (Pb, Cr, Cu, Cd, Ni, As) at different time intervals, as is depicted in fig 2(a), 3(a), 4(a), 5(a), 6(a), 7(a). The growth of the organism gets decreased after exceeding 48 hours of incubation.
As is observed in fig 2(b), 2(c), 2(d), the lowest concentration of Pb content was recorded by Pa which was preceded by MI. Pa and MI contributed to the fast and maximum removal of Pb content. The order of uptake Pb content is as follows: Pa>MI>Bt. The maximum amount of Pb uptake was occurred by Pa. After 48 hours incubation of five isolates in media augmented with 100 ppm of Cr, it was seen in fig. 3(b) that the lowest concentration of Cr was observed by Bt which is preceded by Pm. So, the maximum percent of Cr removal as well as maximum uptake of Cr was recorded by Bt and Pm respectively (Fig 3(c) and Fig. 3(d)). The order of Cr uptake was as follows: Bt>Pm>Bc>Pa>MI.
Cu absorption potentiality was also observed for all selected isolates. As depicted in fig 4(b), the minimum concentration of Cu content in the solution was recorded by MI, and the highest percentage of Cu removal was achieved by MI isolate (Fig 4 (c)). In fig 4(d), the lowest uptake of Cu content was found in Bt whereas the highest percent of Cu uptake was occurred by MI. The order of Cu uptake was as follows: MI>Bc>Pa>Pm>Bt.
Figure 5(a) represents the growth pattern of selected isolates in media supplemented with 100ppm of Cd. The lowest concentration of Cd was observed by Pa after 48 hours of incubation of five isolates. So, the maximum percent of Cd removal as well as maximum uptake of Cd content was obtained by Pa which was followed by Pm isolate. The order of Cd uptake by bacterial isolates was as follows: Pa>Pm>MI>Bt>Bc.
Furthermore, after adding 100ppm Ni in nutrient broth media, all isolates had been examined for Ni concentration after 48 hours of incubation. As is observed in fig 6(b), the highest Ni content was recorded in solution inoculated with Pm, whereas,

the lowest Ni content was observed by *Bacillus cereus*. Among five isolates, *Bacillus cereus* exhibited not only the highest percentage of Ni removal but also the maximum uptake of Ni content (Fig 6(c) and Fig 6(d)). Each isolate's order of uptake potentiality is as follows: Bc> Pa>Bt> MI> Pm.

In our current study, to determine the absorption efficacy of isolates, As (Arsenic) content was estimated in mg/ml after 48 hours incubation of those isolates in a solution containing 100ppm As (Fig 7(b)). As content had shown lowest in solution with Pm isolate; thus contributing to the maximum percent of As removal (Fig 7(c)). Furthermore, according to Fig. 7(d), the efficacy of As uptake can be ordered as follows: Pm>Bt>Pa>Bc> MI.
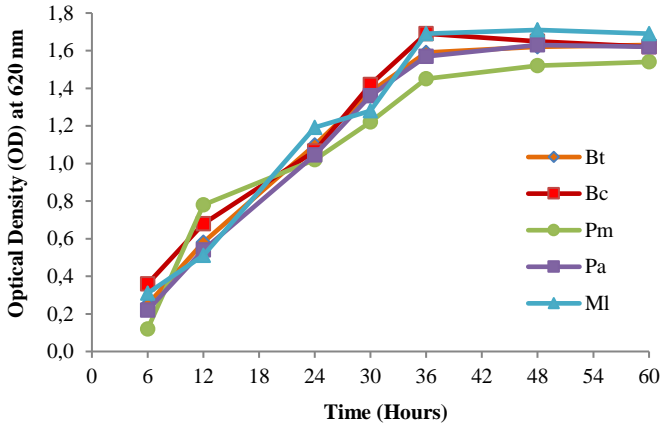
Figure 2 (a): Growth pattern of 05 selective isolates in nutrient broth media with 100 ppm Pb
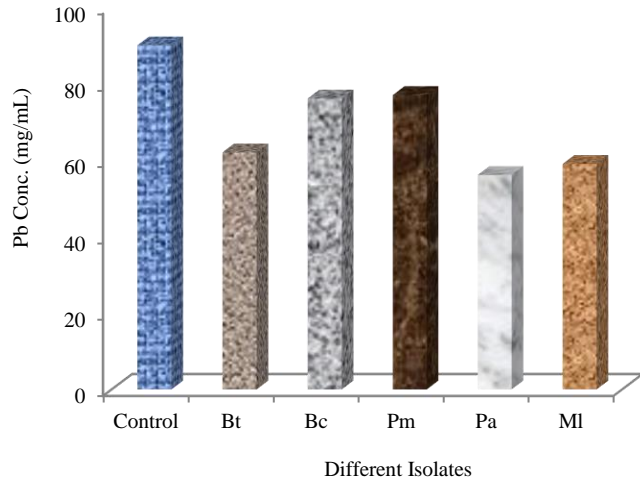
Figure 2 (b): Pb content after 48 hours' incubation by different isolates
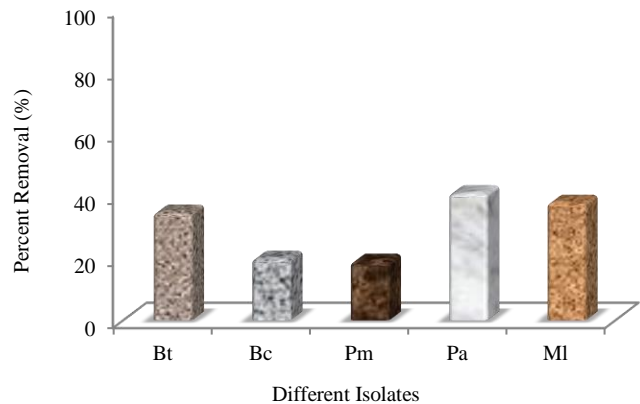
Figure 2 (c): Percent Removal of Pb content by each bacterial isolates
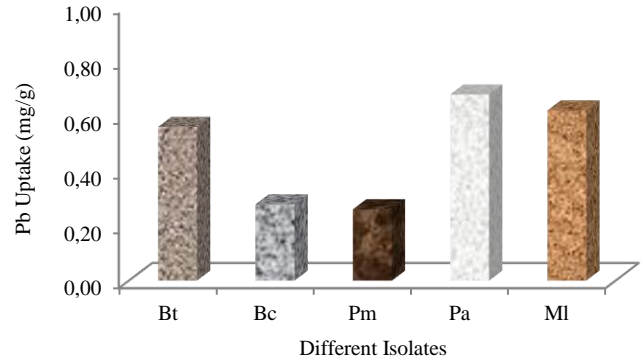
Figure 2 (d):Uptake of Pb content by each bacterial isolates
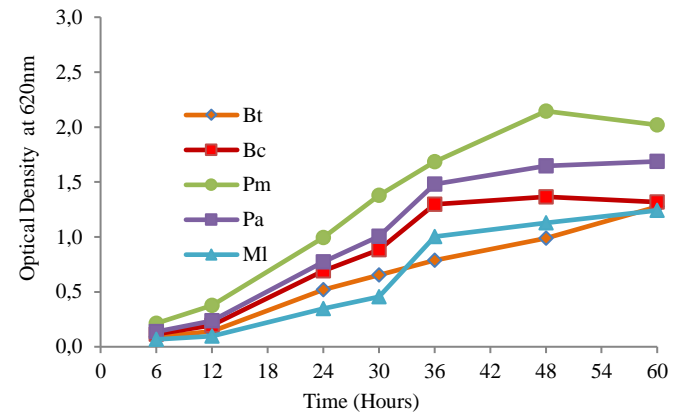
Figure 3 (a):Growth pattern of 05 selective Isolates in nutrient broth media with 100 ppm of Cr
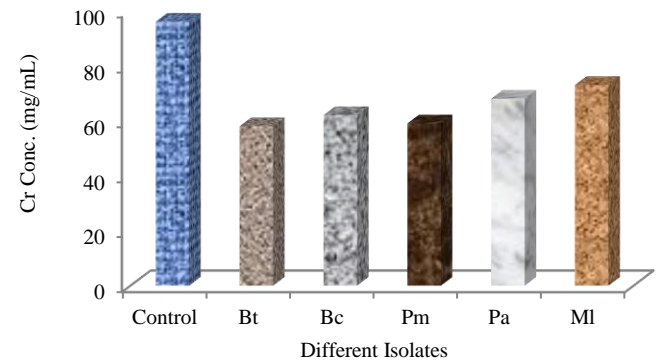
Figure 3 (b):Cr content after 48 hours incubation by different isolates
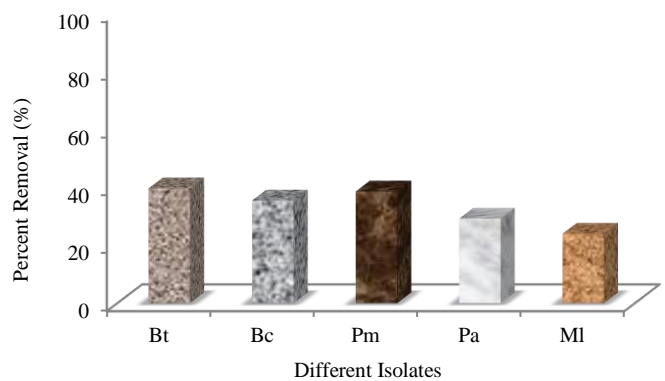
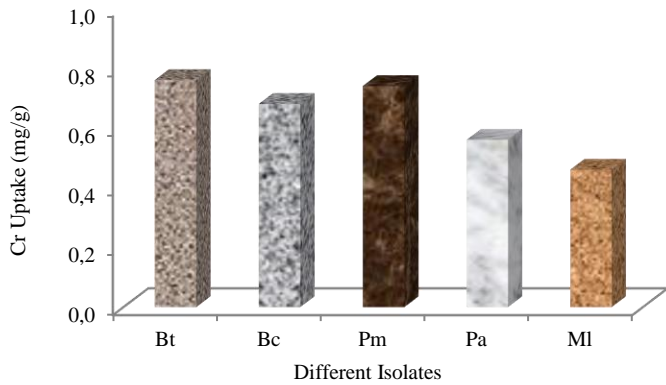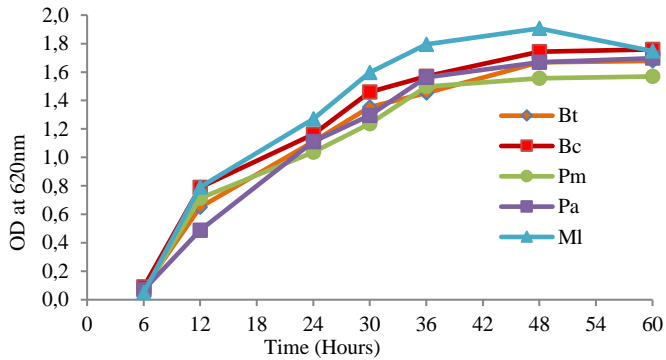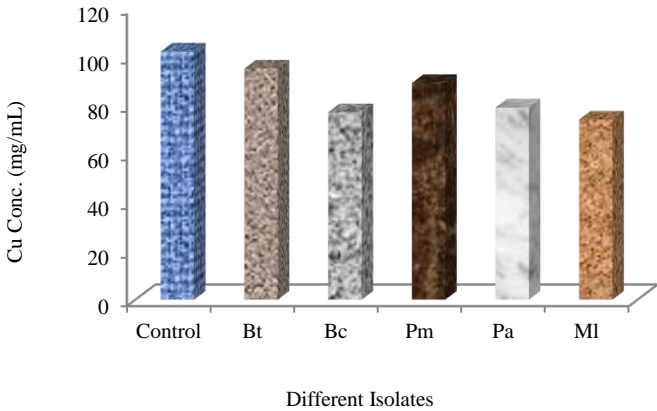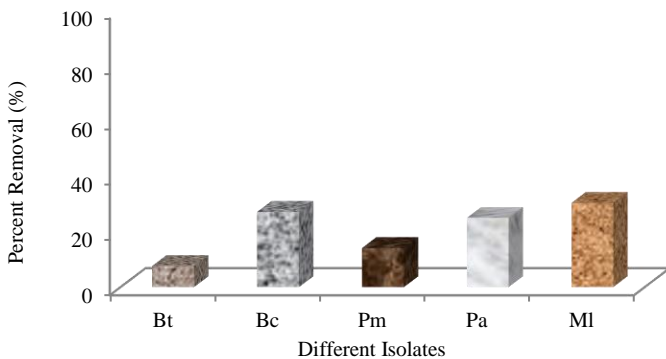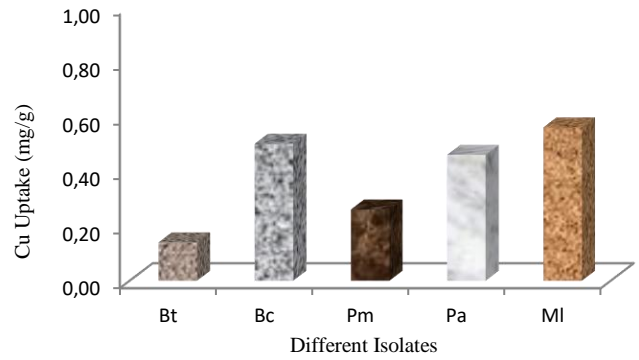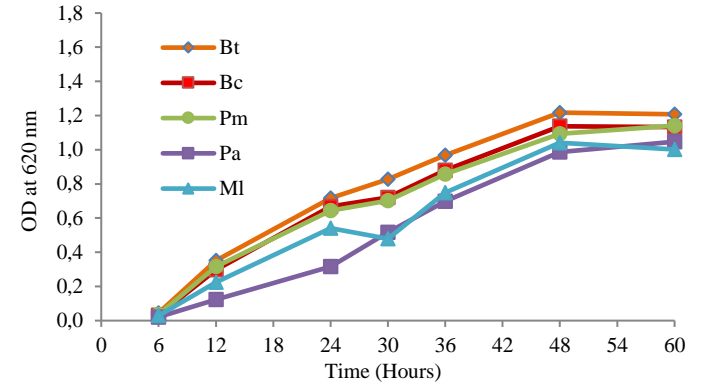**Figure 3** (c): Percent Removal of Cr content by each bacterial isolates

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Figure 3 (d): Uptake of Cr content by each bacterial isolates

Figure 4 (d): Uptake of Cu content by each bacterial isolates

Figure 4 (a): Growth pattern of 05 selective Isolates in nutrient broth media with 100 ppm of Cu.
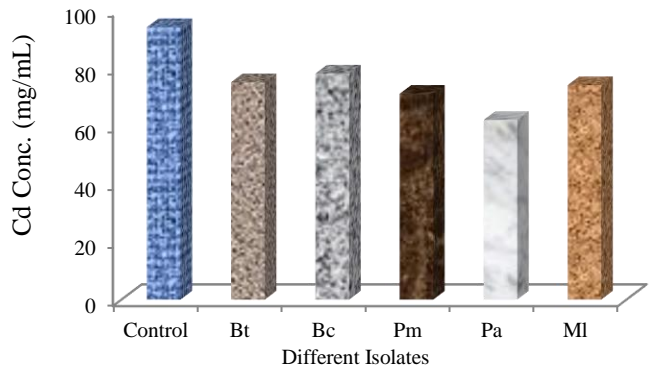

Figure 5 (a): Growth pattern of 05 selective Isolates in nutrient broth media with 100 ppm of Cd.

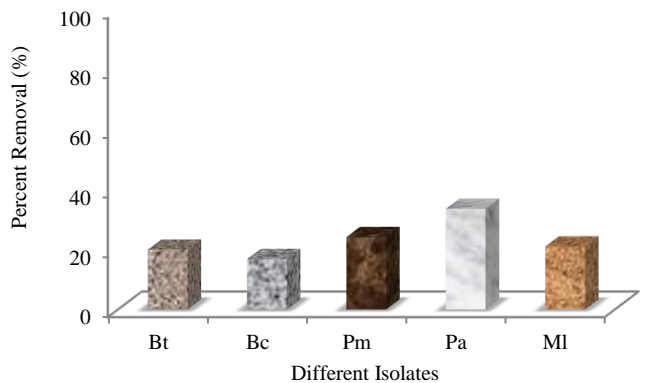**Figure 4** (b): Cu content after 48 hours incubation by different isolates

Figure 5 (b): Cd content after 48 hours incubation by different isolates

Figure 4 (c): Percent Removal of Cu content by each bacterial isolates

Figure 5 (c): Percent Removal of Cd content by each bacterial isolates

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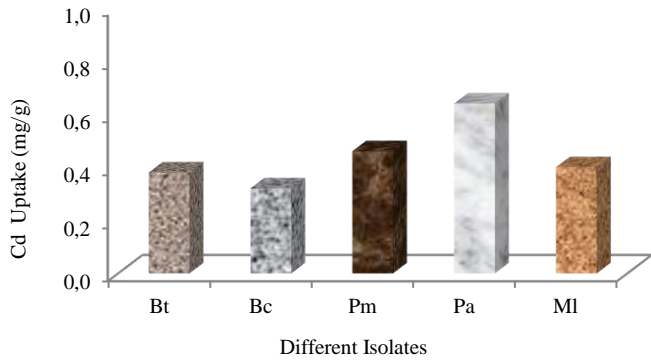**Figure 5** (d): Uptake of Cd content by each bacterial isolates

Figure 6 (d): Uptake of Ni content by each bacterial isolates

Figure 6 (a): Growth pattern of 05 selective Isolates in nutrient broth media with 100 ppm of Ni.
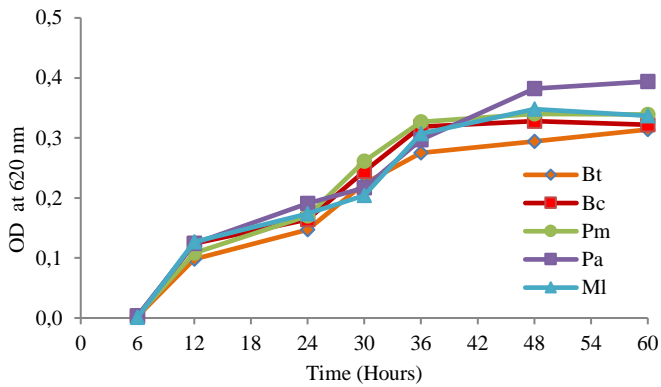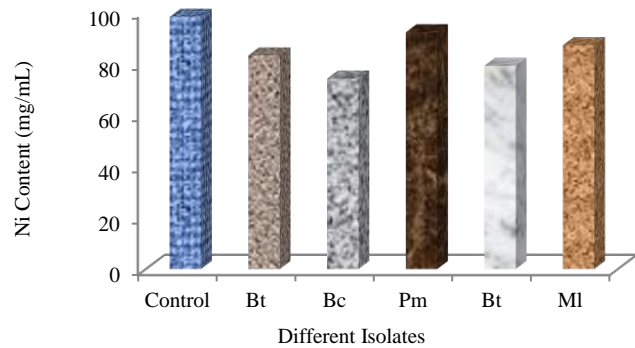

Figure 7 (a): Growth pattern of 05 selective Isolates in nutrient broth media with 100 ppm of As.

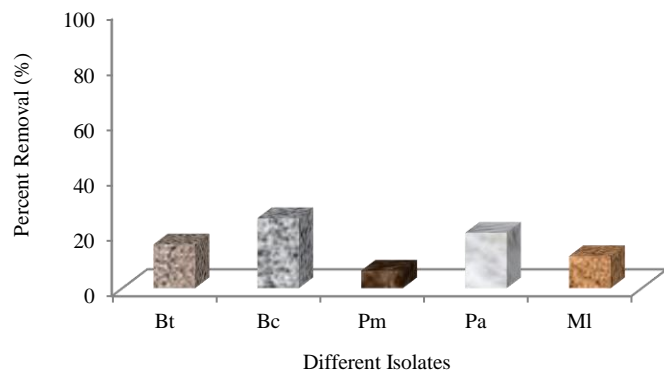**Figure 6** (b): Ni content after 48 hours incubation by different isolates

Figure 7 (b): As for content after 48 hours incubation by different isolates

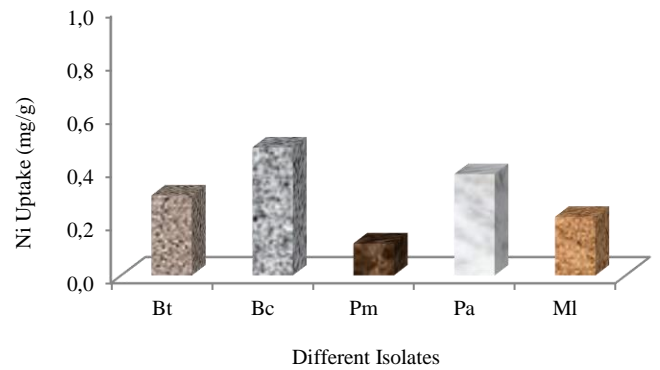**Figure 6** (c): Percent Removal of Ni content by each bacterial isolates

Figure 7 (c): Percent Removal of As content by each bacterial isolates

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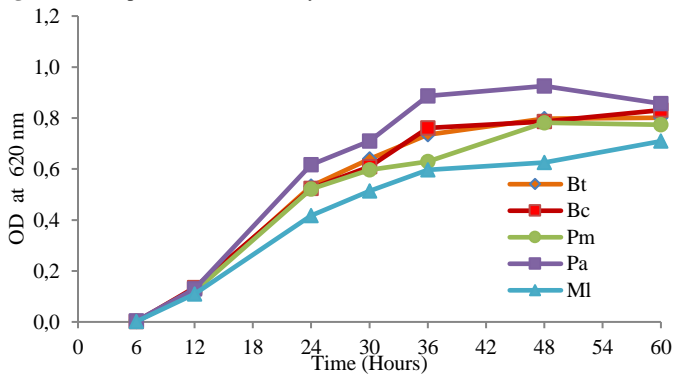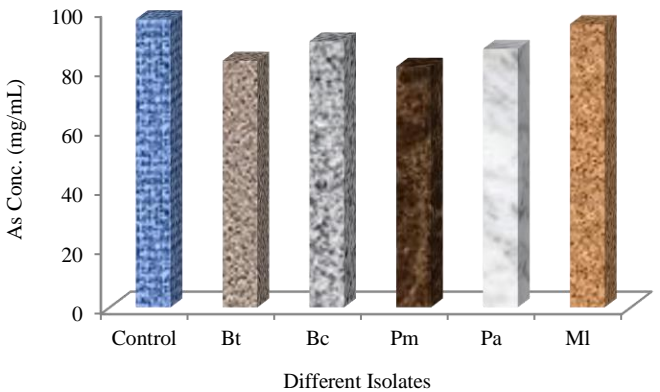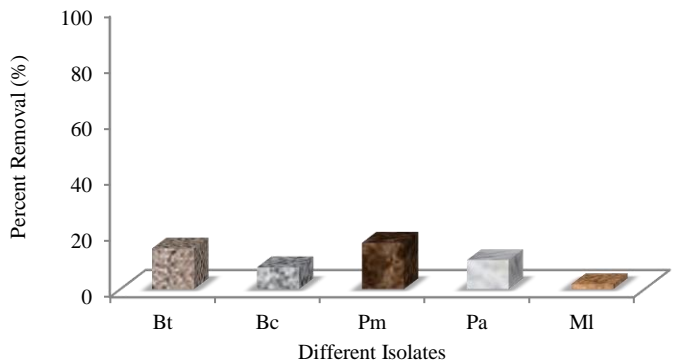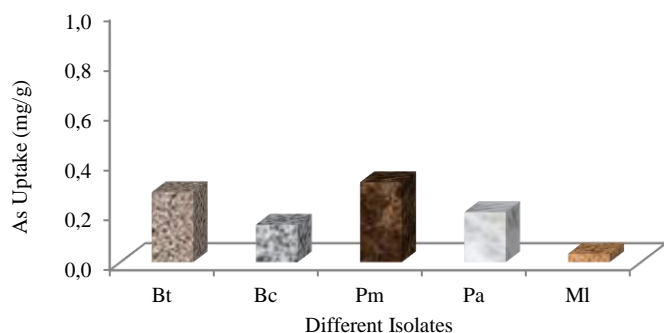**Figure 7** (d): Uptake of As content by each bacterial isolates

DISCUSSION

The preliminary aims of metal remediation are the removal of toxic metal elements from waste or mitigating the toxicity of metals from samples. A plethora of microbial-triggered reactions can fulfill these objectives through oxidation-reduction reactions, metal methylations, and metal complexation **(Kumar *et al*., 2010)**. Various types of microbial metabolic activities have long been manipulated for the welfare of human beings, such as in bioleaching to extract valuable metals from ores. The relationships between metals and microbes have contributed greatly to the progress of bioremediation **(Malik, 2004; Bruins *et al*., 2000)**. Biosorption also called the removal of metal as a biological process that offers great advantages over traditional processes. It is easier to handle and more efficient, non-polluting, and can be incredibly selective; hence making it very cost-effective for treating huge volumes of wastewater with a low concentration of metals **(Puranik and Pakniker, 1999)**.

Different microbes that are resistant to various metal molecules can grow and spread exponentially with the help of industrial wastes. The investigation of several metals-tolerant bacteria could be a useful method for simultaneously monitoring numerous harmful pollutants in an environmental sample. It is noted that both household and industrial wastes contribute to the resistance of bacteria that results in the threat to the environment and human health **(Hossain and Anwar, 2012)**. Since all tested isolates showed significant tolerance against each heavy metal used **(Islam *et. al.,* 2020)**. Therefore, the bacterial species used in this experiment could be a better choice as a biosorption tool to treat effluents from industries that handle toxic heavy metals.In this experiment, the biosorption capacity of identified strains for Cd^{2+} ranged from 16.8% to 33.7%, Cr^{6+} ranged from 23.96% to 39.58%, Ni^{2+} ranged from 6.3% to25.3%, Cu^{2+}ranged from 7.61% to30.43%, Pb^{2+} ranged from 18.95% to 40.95%, and As^{5+}ranged from 1.75% to16.49%.
According to **Nanda *et al.,* (2011)**; *Bacillus sp* and *Pseudomonas sp*., were capable of reducing Cd from tannery effluent samples with an overall reduction of 44% and 56% respectively, after treatment. *Pseudomonas sp.* was proven to be capable of average reducing 34% As,Cu was removed by both *Bacillus sp.* and *Staphylococcus sp.* with an average reduction of 62% and 34% respectively. Similar results were obtained in terms of *Trametes versicolor* for the removal of Pb^{2+} and Cu^{2+} **(Bayramoglu *et al.,* 2003)**, *Bacillus sp*. for chromium (Cr) removal **(Wang *et al.,* 1995)**, *Bacillus firmus* for lead (Pb) and copper (Cu) **(Salehizadeh, 2003)**.
Pseudomonas sp. removed the heavy metals of Co, As and Cd, while *Bacillus sp.* Removed the heavy metals Hg and Cu. According to **Nanda *et al.,* (2011)**, the average Cd removal was 56% with average As removal by 34%, and average Co removal was reported as 53% by *Pseudomonas sp*. On average, 45% of mercury (Hg)and 62% of Copper (Cu)were reduced by *Bacillus sp.*
Several parameters influenced the biosorption ability of *Klebsiella pneumonia* that including the contact period, the initial lead concentration, the pH of the lead solution, and the amount of lead used **(AL-Garni, 2005)**.

CONCLUSION

Heavy metal biosorption by different microbial biomass has become a crucial option for the removal of heavy metals.In the present study, five microbial strains *Bacillus thuringiensis, Bacillus cereus, Pseudomonas monteilii, Pseudomonas aeruginosa,* and *Micrococcus luteus* were screened for biosorption study. After treatment processes, *Pseudomonas aeruginosa* reduced the highest amount of Pb was 0.68mg/g (41%), *Bacillus thuringiensis* also reduced the huge amount of Cr

to 0.74mg/g (39%) respectively. The result showed that the highest degradation of Ni and Cu were shown by *Bacillus cereus* o.48mg/g (25%) and *Micrococcus luteus*that was around 0.56mg/g (30%) and moderate degradation of Cd was shown by *Pseudomonas monteilii* that was around 0.46mg/g (24%). The entire bacterial strains uptake a relatively lowest amount of As that range from 0.03-0.32mg/g and percent removal was 2-16%.

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