Determination of Minimum Inhibitory Concentration of Chromium Salts on the Microbial Strains Isolated from Buriganga River-Bed Soil

By

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A thesis submitted to the Department of Pharmacy in partial fulfillment of the requirements for the degree of
Bachelor of Pharmacy (Hons.)

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The thesis/project titled “Determination of minimum inhibitory concentration of Chromium salts on the microbial strains isolated from Buriganga river-bed soil” submitted by Farzana Ishrat Zaman (16146020) of Spring, 2016 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.) on October 2020.

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Abstract

The presence of heavy metal in the environment is currently increasing in amount especially in soil and water due to their frequent use. Most of the heavy metals are harmful and toxic. One of the heavy metals is Chromium which is abundantly found in mining, tannery, dye industries. The usage of different techniques in removal of heavy metal have increased due to its exposure. Minimum In this experiment, 14 different bacterial strains were used against different chromium concentration analyzed their resistance capacity towards Chromium. Bacterial growth was first observed in media and their minimum inhibitory concentration were measured in chromium concentrated environment. Minimum inhibitory concentration is the lowest concentration of an anti-microbial that resist the growth of a microorganism. The purpose of the study was to identify heavy metal resistant bacterial strains by the minimum inhibitory concentration test.

Keywords: Chromium; MIC; heavy metals; bacterial growth.

Dedication

I dedicate this work to my parents

Acknowledgement

First of all, I want to show my gratitude to Almighty Allah for giving me the strength and motivation to complete this project and overcome all the obstacles that accompanied with it. Without his blessings, I would never be able to do all the works regarding my project.

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List of Acronyms

MIC	Minimum Inhibitory Concentration
EFSA	European Food Safety Authority
Cr (IV)	Hexavalent Chromium
ROS	Reactive Oxygen Species
NB	Nutrient Broth
NA	Nutrient Agar
UV-Vis	Ultra Violet Visible
mM	Milli-molar
mL	Milliliter
µg	Micro-gram
Mg	Milligram

Chapter 1

Introduction

1.1 Background

Heavy metals are the natural element found in the atmosphere. They are termed as heavy metals because they tend to have high density relative to other elements and are relatively poisonous or toxic at low concentration. Some of the heavy metals include- Cadmium (Cd), Chromium (Cr), Mercury (Hg), Cobalt (Co), Arsenic (As), Lead (Pb), Selenium (Se) etc. Heavy metals cannot be destroyed or degraded. To a small extent, some of the heavy metals are important for metabolism for human body. But most of the cases they are harmful for human as well as environment at higher concentration. Due to industrialization, in recent year there has been immense increase in using heavy metals. Heavy metals have various industrial usage because of their several technological significance (Zahoor & Rehman, 2009). Since metals appear to accumulate, they are dangerous. Such metallic elements are known to be a systemic toxic material and are capable of causing significant damage to organ even at lower concentrations. Moreover, in accordance with the U.S. Environmental Protection Agency, and the International Agency for Research on Cancer heavy metals are considered as human carcinogens (Tchounwou, Yedjou, Patlolla, & Sutton, 2012). Heavy metals can come from sources that are natural and anthropogenic. The heavy metals from the natural sources are: volcanic eruptions, weathering of rocks, sprays of sea-salt, biogenic sources, forest fire and solid airborne particles. Heavy metals are natural elements which exist in the earth's crust but, most environmental pollution and exposure to human are occurred by anthropogenic activities, for instance – smelting and mining operations, industrial generation of

heavy metal and its uses as well as agricultural and domestic use of metals and metalloids (Tchounwou et al., 2012).

To decrease the toxicity of the heavy metals many processes such as- oxidation/reduction, membrane technology, reverse osmosis, filtration, electrochemical treatment, chemical precipitation has been followed (Guo et al., 2010). Bioremediation is one of the cost-effective processes. Bioremediation is referred as a natural process involving biological agents, primarily micro-organisms such as - bacteria, plants, algae, yeast and fungi to minimize metal contamination, remove toxic waste and reduce pollution of the environment. It mainly works by transforming the heavy metal in less harmful state and reduce their toxicity. While most of the heavy metal reduction process are costly, bioremediation is cost-effective and environment friendly. They are applied in large scale in soil, waste water, and industrial waste. Using micro-organisms with heavy metal resistance properties, it will be easier to integrate them into the cycle of bioremediation, where they can help reduce metals from a specific region.

This study is designed to estimate the minimum inhibitory concentration of chromium through using microorganisms. The analyses will be based upon the chromium resistance property in the applied bacterial strain.

1.2 Methodology

The study was conducted to determine the minimum inhibitory concentration of chromium in isolated bacteria from Buriganga river bed soil. 14 different isolated bacteria were experimented against the chromium and their growth in different chromium concentration was observed. Nutrient broth was used as the bacterial growth culture media. Saline water was used for dilution of the bacteria to ensure the viability of the isolated bacteria. Different increasing concentration of chromium was taken to see the growth pattern of each bacterium. Finally, absorbance was taken

to observe the growth which was determined as the minimum inhibitory concentration of chromium at a particular concentration.

1.3 Aim and objective of the study

The hypothesis of this project was to check the chromium resistance pattern of some isolated bacterial strains. The objectives of this project are:

1. Measurement of the minimum inhibitory concentration chromium by the bacterial strains
2. Analyze the growth pattern of the bacterial strains in chromium rich environment.

1.4 Literature review

Heavy metals are the trace elements found in earth crust. They are the metals and metalloids which usually have the density higher than $4g/cm^{-3}$ (Paul, 2017). There are natural and anthropogenic sources available for heavy metals. Industry based sources are more prone to cause harm to human and environment. Some of the industrial sources of heavy metals are listed in table below.

Table 1: Sources of different heavy metals (Paul, 2017)

Heavy Metals	**Sources**
Cadmium (Cd)	Electroplating, welding, fertilizer, pesticides, nuclear fission plant, batteries
Arsenic (As)	Fungicides, pesticides metal smelters
Chromium (Cr)	Tannery, electroplating, mining, textile industries
Mercury (Hg)	Paper industries, batteries, pesticides

Copper (Cu)	Electroplating, pesticides, mining
Lead (Pb)	Pesticides, paints, batteries, emission automobile, burning of coal, mining
Nickel (Ni)	Zinc base casting, electroplating, battery industries
Zinc (Zn)	Brass manufacture, refineries, metal plating, immersion of painted idols
Manganese (Mn)	Fuel addition, electroplating, ferromanganese production

Heavy metal hazards are seen in human when the daily intake level exceeds the maximum tolerant level. Daily intake of heavy metals mostly depends on the heavy metal exposure, daily food intake and concentration of heavy metal in the food and air. Estimated daily intake (EDI) of heavy metal is calculated by (Asomugha et al., 2016):

$$EDI = \frac{C_{metal} \times D_{food\ intake}}{BW_{average}}$$

Where:

C - The concentration of chromium in mg/kg

D - The daily food intake in kg person^{-1}

BW - average body weight in kg person^{-1}

1.4.1 Heavy metals toxicity in human

Intake of heavy metals in human through air and food must not exceed the maximum tolerance level. Different regulatory bodies have given maximum tolerable concentration of heavy

metals in human. Given table 2 represents maximum tolerable concentration of chromium according to the regulatory body.

Table 2: Tolerable daily intake of heavy metal (Asomugha et al., 2016)

Heavy metals	**Concentration (mg/kg day^{-1} BW)**	**Regulatory body**
Nickel	0.0028	EFSA,2015
Lead	0.0005	WHO, FAO 2010
Chromium	0.3	EFSA, 2014
Cobalt	0.023	FSA,2003
Zinc	0.43	SCF, 2003
Iron	0.8	EFSA, FAO/WHO, 2010

If the daily intake of the heavy metals goes beyond the prescribed value recommended by the regulatory bodies, they can cause significant harm to the human health. For instance, liver and kidney damage, sickle cell anemia, carcinogenicity, arrhythmia, gastrointestinal failure etc. are associated with heavy metals (Järup, 2003). Heavy metals in exposure to human cells turn into free radicals which is harmful for the human body.

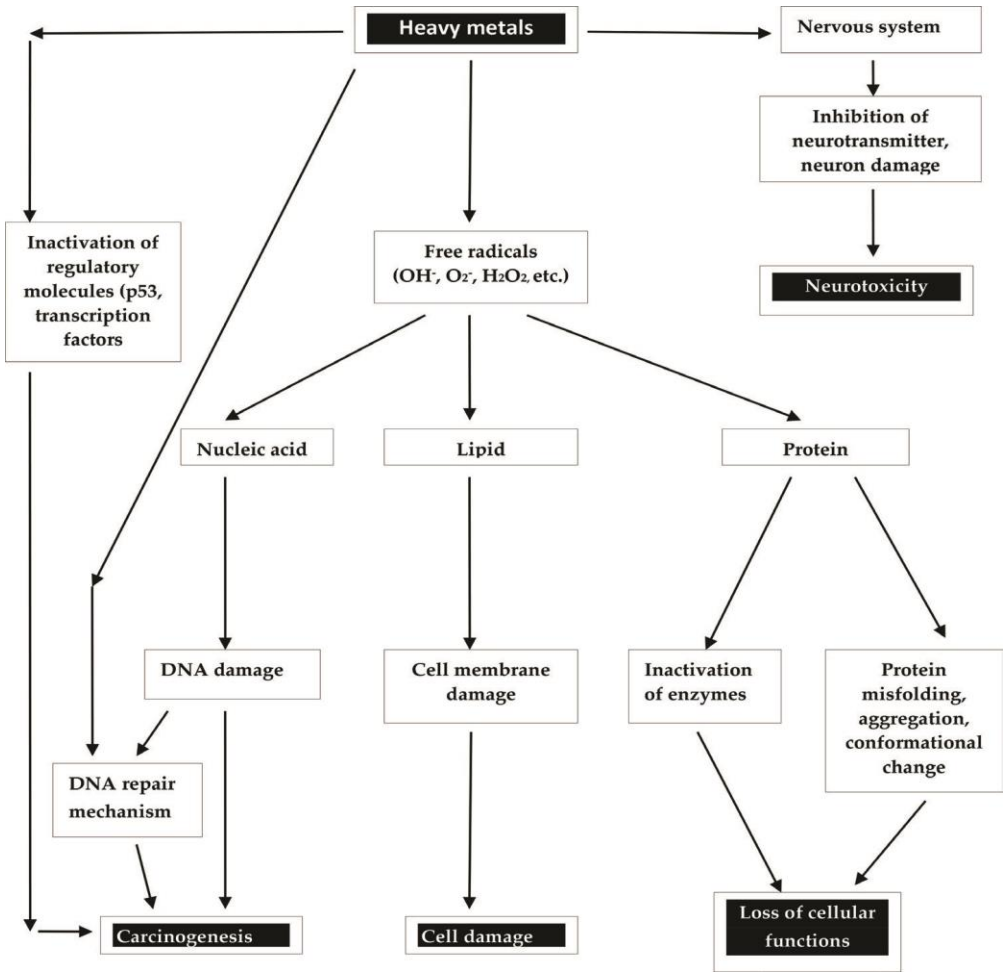

Figure 1: Network of heavy metal toxicity in human cell (Drahansky et al., 2016)

At high concentration, most of the heavy metal show toxic effect. Various harmful outcomes of these heavy metals on human body are discussed on the table below.

Table 3: Harmful effects of heavy metal on human health

Heavy metals	**Harmful effects**	**Reference**
Mercury	Anxiety, depression, visual disturbance	(Sarwar et al., 2017)
Cadmium	Carcinogenicity, renal failure, kidney damage	(Torres-Martínez, Kharissova, & Kharisov, 2019)

Lead	Mental retardation, irritation, brain damage	(Awokunmi, Asaolu, Adefemi, & Gbolagade, 2015)
Nickel	Neurotoxic, genotoxic, hepatotoxic	(Sarwar et al., 2017)
Copper	Liver and kidney damage, liver cirrhosis, intestine irritation	(Wuana & Okieimen, 2011)

1.4.2 Biochemistry of heavy metal toxicity

Heavy metals show poisonous effect due to their interference with the biochemical networks of human body. The oxidative states of heavy metals are reduced in the cytoplasm due to different oxidizing enzymes which are stable in nature and bind with biological protein and enzyme to create complex bond (Duruibe, J. O., Ogwuegbu, M. O. C. and Egwurugwu, 2007). The reaction below shows their oxidative state after binding with protein or enzyme with the sulphydryl groups (-SH) of cysteine and sulphur atoms of methionine (-SCH_3) consecutively (Duruibe, J. O., Ogwuegbu, M. O. C. and Egwurugwu, 2007).

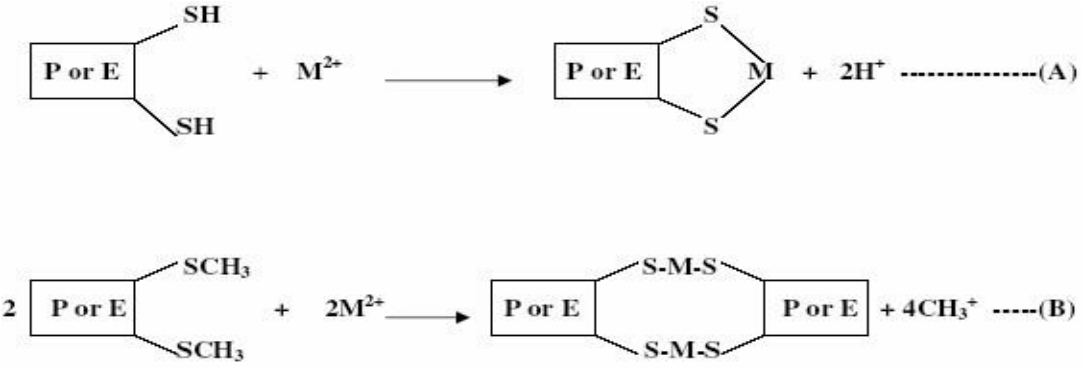

Figure 2: (A) Intramolecular bonding and (B) Intermolecular bonding of protein, enzyme and metal (Duruibe, J. O., Ogwuegbu, M. O. C. and Egwurugwu, 2007)

Where, P = Protein; E = Enzyme; M= Metal

Here, the hydrogen group of the metals turns into poisonous compound thus inhibiting enzymes from function and the protein- metal compound reacts with the metabolic enzymes. The reaction takes place either in induced fit pattern or lock and key pattern to form an enzyme-substrate complex. This inhibits the enzyme to binds with any other substrates and no bioreaction can take place.

1.4.3 Heavy metal resistance in bacteria

Many microorganisms need exposure to heavy metals for proper growth. But heavy metals at higher concentration also disrupt bacterial growth. However, some of the bacteria may evolve and gain the protection mechanism against heavy metals which ensure their survival at higher concentration of heavy metals. Just like antibiotic resistance, bacteria can become heavy metal resistant as well. Five mechanisms of bacterial resistance against heavy metals have been identified. A single bacterium can acquire more than one resistance mechanism. The mechanisms are mentioned below (Ianeva, 2009):

- Extracellular barrier
- Active transport of metal ions (efflux)
- Extracellular sequestration
- Intracellular sequestration
- Reduction of metal ions

1.4.4 Characteristics of Chromium

Chromium is available in trace amount in the environment. It is used as the metal alloys in chrome plating, metal ceramics, and stainless-steel industry. They are also used in dyes, paints etc. Because

the usage of chromium in industries, people working in those industries are heavily exposed to its toxicity. Chromium has many oxidative states. Among them the most stable states of chromium are – trivalent chromium, Cr (III) and hexavalent chromium, Cr (VI) (Oliveira, 2012). While Cr (III) is useful for human, Cr (VI) is hazardous. Health hazards related to chromium is commonly observed people living near these metal industries. Development of compatible process for removal of chromium contaminated soils is an important issue as it is related to the environmental protection and recovery, particularly processes which works for the removal of Cr (VI)-contaminated soils (Polti, García, Amoroso, & Abate, 2009).

1.4.4.1 Mechanism of chromium toxicity

As mentioned earlier, hexavalent chromium Cr (VI) is more harmful than trivalent chromium Cr (III). Hexavalent chromium is a strong oxidizing agent. Cr (VI) easily penetrates through the cell membrane through the isoelectric and isostructural SO_4^{2-} and HPO_4^{2-} passages (Jaishankar, Tseten, Anbalagan, Mathew, & Beeregowda, 2014). The chromates ions are then gone through phagocytosis. Reduction of Cr (VI) into Cr (III) in intracellular region is considered to be detoxification process if occurred away from target site. However, if occur in the target site, it activates Cr (VI). The reaction between biological substance such as- thiol specially glutathione, ascorbate and Cr (VI) leads to the production of reactive oxygens including- hydrogen peroxide, superoxide ions and hydroxyl radicals (Stohs & Bagchi, 1995). This results in increase of oxidative stress in intracellular region causing damage to protein and DNA.

Also, studies have shown that hexavalent chromium causes mutation of mammalian cell as well as bacteria. Also, Cr (VI) causes chromosomal damages which may lead to carcinogenicity. Which is why, International Agency for the Research on Cancer have marked Cr (VI) group 1 human carcinogen.

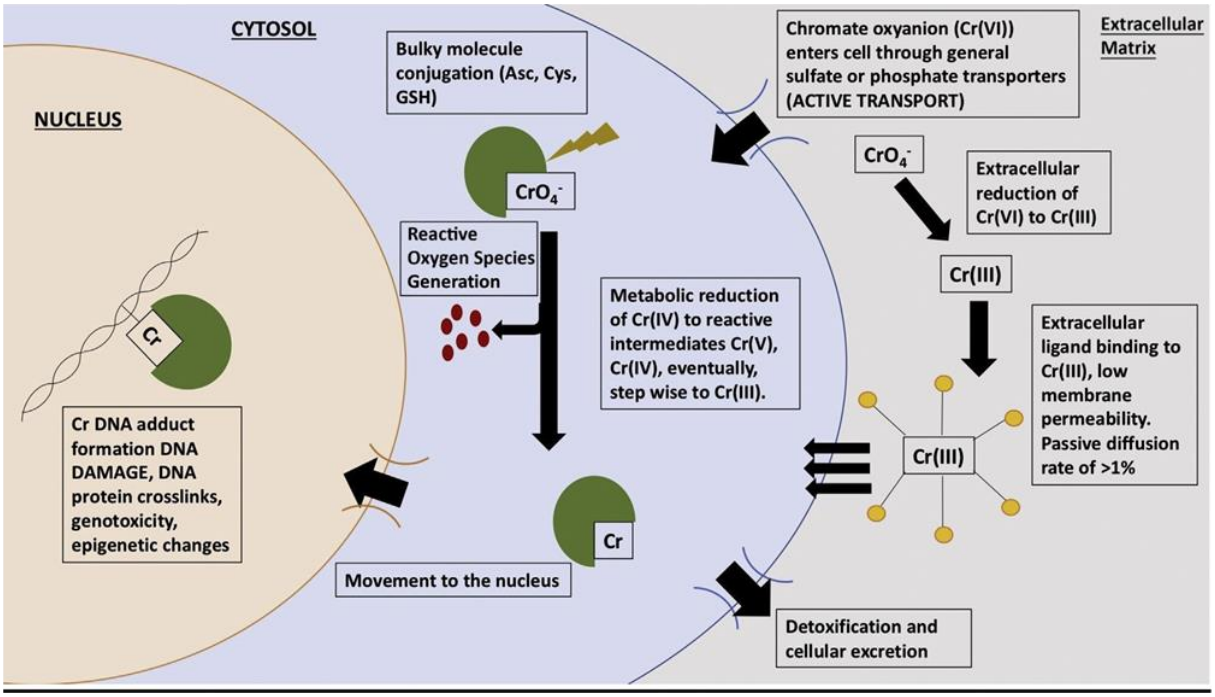

Figure 3: Mechanism of chromium uptake and toxicity (DesMarias & Costa, 2019)

1.4.4.2 Harmful effects of Chromium

Chromium in very small amount is needed for human body. However, being one of the major heavy metals, chromium especially Cr (VI) shows its harmful effect in human, animals and plants at high concentration. In human, chromium causes damage to kidney, respiratory tract, shows carcinogenicity, genotoxicity.

1.4.4.2.1 In human

The harmful effects of chromium on human health mainly depend on organ exposure and dose. Acute reaction occurs when the chromium level goes above 0.3 mg/kg body weight daily. The effects of chromium in different organ system of human body are described in table 4 given below.

Table 4: Harmful effects of chromium on human health

Human health effects of chromium	Interpretation	References
Respiratory tract	Depending on dose, airway obstruction and irritation occurs; asthma, chronic bronchitis, chronic rhinitis, chronic pharyngitis. hyperemia, ulceration of nasal mucous membrane occurs due to pulmonary inhalation	(Zhang et al., 2011) (Gorchev & Ozolins, 2004)
Skin	Acute effect is seen by irritation and dermatitis including fissured skin, swelling, papules, erythema, dryness due to allergies	(Achmad, Budiawan, & Auerkari, 2017) (Zhang et al., 2011)
Cancer	Chromium is associated with cancer in respiratory tract mainly in nasal and lung cancer; the radicals of chromium reaction causes mutation and DNA damage; also, Cr (IV) is find to be inhibit tumor suppressor gene	(Zhang et al., 2011) (Gad, 1989)

1.4.4.2.2 In animals

Chromium affects animal system in a same way like it affects human body. Although it is an important trace element, it can cause toxicity if excessive exposure occurs. When Cr (VI) enters

the cell, it is reduced enzymatically as well as non-enzymatically producing lower valency intermediates resulting in reactive oxygen species (ROS) generation (Malik, Singh, Thakur, Kaur, & Nijhawan, 2016). It causes DNA damage in structural and functional way leading to cellular toxicity. It also affects the skin, kidney, lungs and reproductive system (Sellamuthu, Umbright, Chapman, Leonard, & Li, 2011). Moreover, Cr (VI) concentration ranging 20-90 mg/L shows toxicity in marine fish.

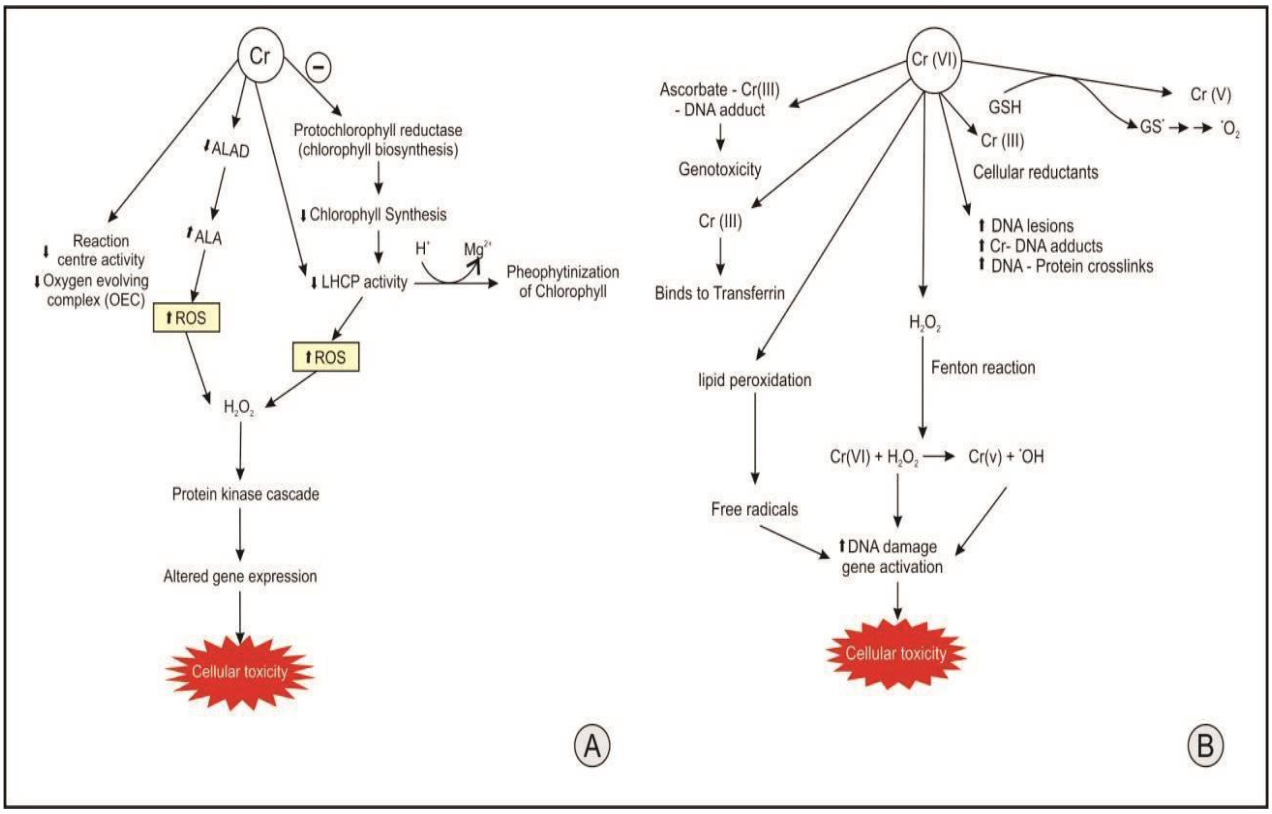

Figure 4: (A) Chromium toxicity in plants, (B) Chromium toxicity in animal (Malik et al., 2016)

1.4.4.2.3 In plants

Cr (VI) is more stable in soil. Due to presence of excessive Cr (VI), germination percentage and bud sprouting of plant are reduced significantly. Also, reduction in root length and plant height is also seen. Reduction in leaf number and number of flowers per plant occurs (Shanker, Cervantes, Loza-Tavera, & Avudainayagam, 2005). Photosynthesis, photophosphorylation and electron

transport system also get affected by presence of chromium (Malik et al., 2016). Chromium stress results in chlorophyll pheophytinization, which decreases plants' efficiency in photosynthesis and increases ROS level.

1.4.4.5 Conventional methods of chromium removal

- Chemical precipitation
- Absorption and biosorption
- Reverse osmosis
- Ion exchange

1.4.4.6 Bacterial mechanism in chromium reduction

In 1977, chromium reducing property of *Pseudomonas* was discovered. After that, many microbes were found to be resistance to chromium. Microbes reducing chromium first have to survive on contaminated environment. There are several mechanisms developed indicating bacterial tolerance against chromium contaminated environment. Those are given below (Cheng, Holman, & Lin, 2012):

(1) ChrA transporter - it is a plasmid encoded transporter which causes efflux of chromate ion from cytoplasm entering the cell by sulfate transporter.

(2) Extracellular reduction of Cr (VI) - Cr (VI) is reduced into Cr (III) that cannot move through the cell membrane. Most bacteria follow this mechanism.

(3) Intracellular reduction to Cr (III) from Cr (VI) - It may cause protein or DNA damage due to oxidative stress. Hence, detoxifying enzymes works as a protection for the cell against oxidative stress as well as reduce the toxicity of chromate ions.

(4) Extracellular barriers or capsules are formed by some bacteria to hinder the influx of Cr (VI) into the cell.

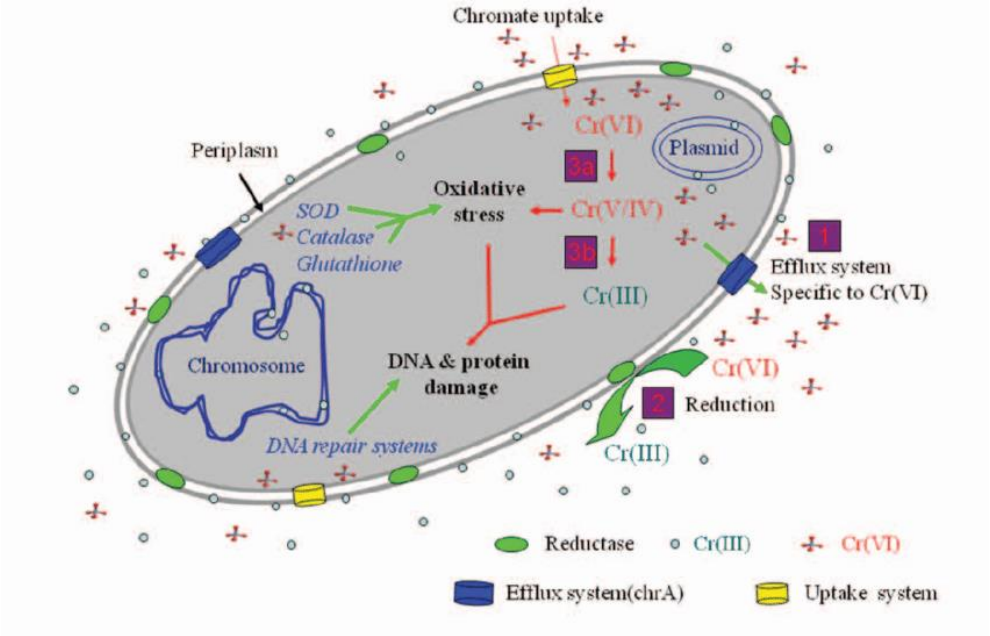

Figure 5: Mechanism of bacterial resistance of chromium (Cheng et al., 2012)

Microbes can follow either enzymatic pathway or non-enzymatic pathway for chromium resistance (Sethunathan N, 2003). In enzymatic reduction, chromate ion is enzymatically reduced while taken up by microbes. Also, the Cr (VI) reductase enzymes are available in the cytoplasm and membrane. The YieF dimer which is a soluble protein found in the cytoplasm, is a four-electron chromate reducer (Ackerley et al., 2003). The dimer is thought to transfer three electrons to chromate and one to molecular oxygen, thus reducing Cr (VI) directly to Cr (III) in one step (Cheng et al., 2012). On the other hand, in non-enzymatic method, microbial metabolism products such as- Fe^{2+} and H_2S help in reduction of Cr (VI) to Cr (III).

Chapter 2

Materials and methods

2.1 Introduction

The experiment was done following standard protocol using standard laboratory chemicals and equipment. In this section, the chemicals, reagents and equipment used in the experiment will be discussed. Also, the procedure of the full experiment such as collection of the sample, different test which was done by the isolated sample, identification of the sample etc. will be discussed in details.

2.2 Chemicals

The list given below shows all the chemical reagents used in the experiment:

1. Nutrient Broth
2. Nutrient Agar
3. Potassium Chromate (K_2CrO_4)
4. Sodium Chloride (NaCl)

2.3 Instruments used

Instruments and glassware that were used in the experiment including their sources are listed in the table 5 given below.

Table 5: Name and function of the instruments

Name of instruments	Function	Manufacturer
Electric balance	Weight measurement	CSC balance, Japan
Autoclave machine	Sterilization	Vision scientific, Korea
Digital Shaking incubator	Incubation of liquid culture mediums	OVAN, Spain
Laminar Air Flow	To maintain the aseptic environment	Labtech, Korea
Sterile syringe filter	To filter the heavy metal salt	China
Samsung refrigerator	For storing bacterial culture stock	Samsung, South Korea
Micropipette	For withdrawing reagent and media in trace amount	Eppendorf, Germany
UV-Vis Spectrophotometer	Measurement of absorbance	Shimadzu, Japan

2.4 Collection of samples

The soil sample containing the bacteria was collected from Buriganga river bed. Buriganga being one of the most polluted rivers in Bangladesh contains high amount of pollutants, heavy metals, wastes etc. Buriganga is situated beside Dhaka, capital of Bangladesh. Within few decades, a lot of industries have been built beside Buriganga River. The industrial wastage and domestic wastes

directly go to the water of the river. There are around 343 tanneries are situated on the Buriganga river bank (Ahammed, Tasfina, Rabbani, & Khaleque, 2015). Every day 40,000 tons of wastewater containing all the toxic and harmful substances is released to the river water (Sarkar et al., 2015). Moreover, human wastes of the city dwellers are also thrown to the river. It is estimated that human waste is responsible for 60% of pollution in the River, industrial waste of 30% and the rest is solid waste (Sarkar et al., 2015). For which the river is now biologically dead. Mostly, The tannery industries that are situated beside the river do not have proper drainage system so all of their wastages are directly released in the river (Mahmood, Nourin, Siddika, & Khan, 2017). The study identified the presence of five heavy metals (Pb, Ni, Cu, Cr and Fe) in the rivers (Atiqur Raman Sunny , S. Naznin, M. J. Rahman, 2017). The pollution can spread to the water, soil, air and then to the environment and can cause severe effect to human health and can also cause acid rain, global warming etc. (Kibria, 2015).

To conduct the experiment, we have collected the soil sample from Showarighat and Pargandaria areas of Buriganga river bank.

2.5 Isolated Bacterial strains

An isolation process was done on the sample to isolate the bacteria. 14 different types of bacteria were previously isolated. The experiment was conducted to identify whether the bacteria are chromium resistant bacteria or not. Also, the analyzation of the minimum inhibitory concentration of chromium to these bacteria was done. Different name tags were given to the 14 bacteria, such as: A1, A2, B1, B2, C1, C2, D1, D2, E1, E2, F1, F2, G1, and G2.

2.6 Pre-MIC test processes

DAY1

All the bacteria were cultured in agar plates. 14 petri dishes, the agar media and the materials involved to inoculate the bacteria in the agar plates were autoclaved to ensure that they are free from any other micro-organisms. 350ml agar media was prepared where 9.8gm of Nutrient agar was mixed with distilled water in a conical flask and it was poured up to 350ml. 1mM of K_2CrO_4 was added in the agar media after the autoclave. After the autoclave was done, agar media was transferred to the petri dishes and all the 14 different bacterial strains were inoculated in the agar plates. They were then kept in the incubator for 48hrs to have a proper growth.

DAY2

After 24hrs the bacterial growth were observed. For better result it can be kept for 48 hrs.

DAY3

After the completion of 48hrs of incubation of the bacteria, another subculture was done following the same way that is previously described. Then it is again kept for 48hrs of incubation in the incubator.

DAY 4

After 24hrs the bacterial growth were observed. For better result it can be kept for 48hrs.

DAY 5

After 48hrs the growth of the subcultures were observed and better growth was seen. Now, after the subculture was done there were in total two culture plates available for each bacterium. The culture that has shown better and clear growth was chosen for the further analysis process.

A nutrient broth solution was prepared where each of the bacteria from the chosen culture plates was inoculated in different broth solution. The total 14 test-tubes containing 14 different bacteria in nutrient broth solution were kept in the incubator for 48hrs. From these bacterial cultures the later MIC tests were done.

2.6.1 Preparation of Nutrient Broth solution

To make the nutrient broth solution, 13g of nutrient broth powder was taken in 1000ml of distilled water. The solution was made according to the instruction written on nutrient broth container. The solution was then sterilized through autoclave machine to make sure no bacteria are present. The broth was further used for the culture of bacterial strain.

2.6.2 Preparation of 0.9% NaCl solution

To prepare the 0.9% NaCl solution, 9gm of NaCl salt was taken in a conical flask and distilled water was added to make 1000ml solution. 0.9% NaCl solution (saline water) was used for dilution of bacterial culture. 10 times dilution was done to make sure there are proper growths of bacteria significant for appropriate result.

2.6.3 Preparation of 1M Heavy Metal salt solution

The molecular weight of the Potassium Chromate (K_2CrO_4) is 194gm. To prepare K_2CrO_4 solution, 1.94gm of potassium chromate was added to 10ml of distilled water for each day use. The heavy metal salt was filtered using syringe filtration to make sure no contamination was present in it. The prepared chromium salt solution was further used to prepare salt containing broth where the minimum inhibitory concentration of the bacterial strains was tested.

2.6.4 Preparation of broth & heavy metal salt solution of different concentration

The solution containing broth and chromium salt was of made up to 15ml in total for different concentration in the conical flask. For each bacterial strain the concentration was measured for 3 times. So, for each concentration 3 test-tubes were taken containing 5ml of chromium salt-broth solution from which minimum inhibitory concentration was measured. Measurements of the ingredients in some of the different concentrations of salts are given table 6.

Table 6: Different concentration of broth and heavy metal salt solution measurement

Concentration	**Amount of heavy metal salt**	**Amount of Broth**	**Total volume of the Solution**
3mM	45µL	14.955ml	15ml
5mM	75µL	14.925ml	15ml
7mM	105µL	14.895ml	15ml
10mM	150µL	14.850ml	15ml
13mM	195µL	14.805ml	15ml
15mM	225µL	14.775ml	15ml
17mM	255µL	14.745ml	15ml
19mM	285µL	14.715ml	15ml
20mM	300µL	14.700ml	15ml
23mM	345µL	14.655ml	15ml
25mM	375µL	14.625ml	15ml
30mM	405µL	14.595ml	15ml

2.7 Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentrations (MICs) can be verified as the lowest concentration of an anti-microbial that will inhibit the visible growth of a microorganism after overnight incubation, and (Andrews, 2001). To determine the MIC, bacteria was incubated in the nutrient broth media at different concentration of Potassium chromate (3mM to 30mM). Before incubation of bacteria, 10 times serial dilution of the bacteria was done in saline water. For each bacteria concentration, three test tubes were taken each containing 5mL of the broth and heavy metal salt solution. 50µL of the diluted bacterial culture in saline was added to each test tube of the solution by using a micropipette. All the test tubes that contained the bacterial culture was kept into the shaking incubator at 37˚C for 24 hours. The next day, absorbance was measured at 600nm of the incubated culture to determine the growth. According to the absorbance, no bacterial growth in a particular concentration was the minimum inhibitory concentration for those particular bacteria.

Chapter 3

Result and Discussion

3.1 Results

3.1.1 Minimum Inhibitory Concentration of Chromium to inhibit the growth of A1 bacteria

The Minimum Inhibitory Concentration of the isolated sample A1 was determined and the obtained result is given below in the following table.

Table 7: Minimum inhibitory concentration of isolated bacteria A1 against different chromium concentration

Concentration (mM)	Test tube 1	Test tube 2	Test tube 3	Average	Standard Deviation
3	0.033	0.024	0.039	0.032	0.006164414
7	0.051	0.021	0.022	0.031333	0.017039171
10	0.016	0.019	0.011	0.015333	0.004041452
15	0.007	0.008	0.005	0.006667	0.001527525
19	0.004	0.004	0.008	0.005333	0.002309401
23	0.001	0	0.001	0.000667	0.00057735
24	0	0	0	0	0

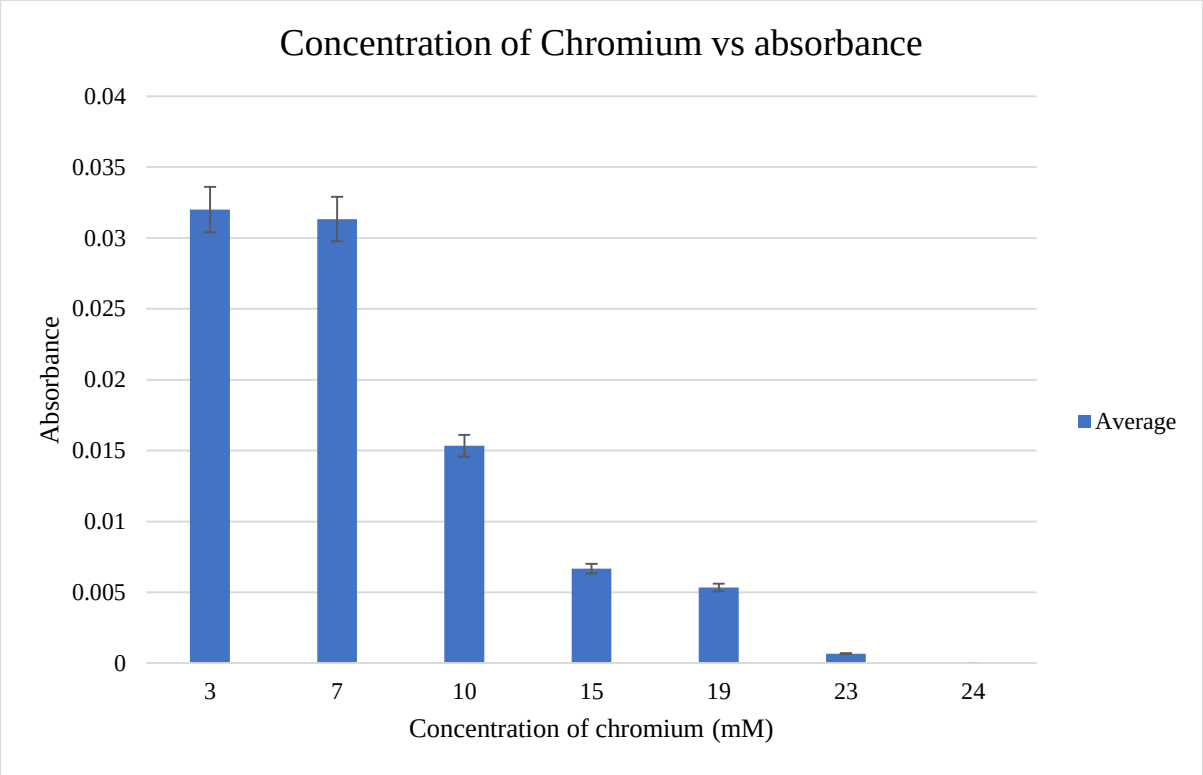

Figure 6: Minimum inhibitory concentration of the isolated sample A1

From the figure, it can be seen that the isolated bacterial strain A1 has the ability to withstand Chromium concentration up to 23mM. Therefore, it can be said that A1 was capable to show resistance till the Chromium concentration of 23mM. However, from 24mM concentration, no growth of A1 was observed. So, the Minimum Inhibitory Concentration for the A1 isolate is 24mM.

3.1.2 Minimum Inhibitory Concentration of Chromium to inhibit the growth of A2 bacteria

The Minimum Inhibitory Concentration of the isolated sample A2 was determined and the obtained result is given below in the following table.

Table 8: Minimum inhibitory concentration of isolated bacteria A2 against different chromium concentration

Concentration (mM)	Test tube 1	Test tube 2	Test tube 3	Average	Standard Deviation
3	0.355	0.353	0.359	0.355667	0.00305505
7	0.249	0.238	0.212	0.233	0.019
10	0.115	0.14	0.199	0.151333	0.043131582
15	0.099	0.097	0.103	0.099667	0.00305505
19	0.064	0.057	0.081	0.067333	0.012342339
23	0.013	0.022	0.026	0.020333	0.006658328
25	0.013	0.011	0.007	0.010333	0.00305505
27	0.002	0.001	0.002	0.001667	0.00057735
28	0	0	0	0	0

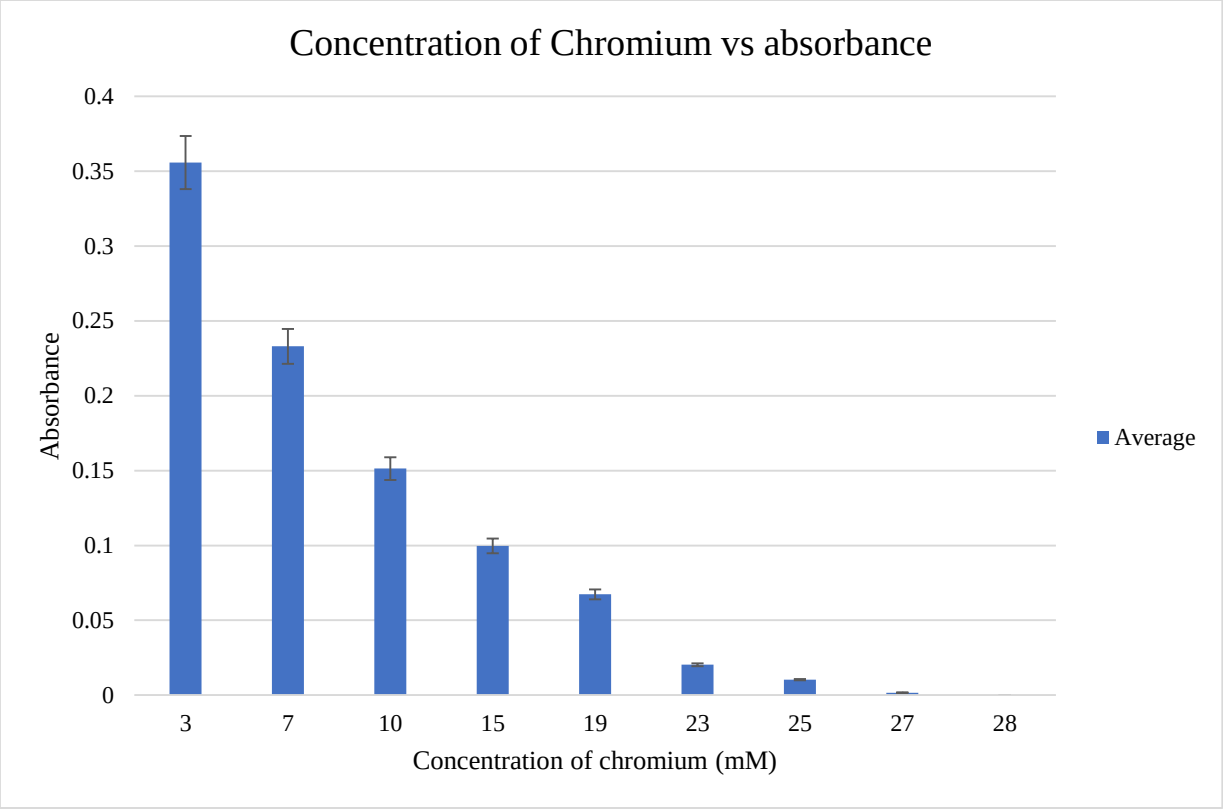

Figure 7: Minimum inhibitory concentration of the isolated sample A2

From the figure, it can be seen that the isolated bacterial strain A2 has the ability to withstand Chromium concentration up to 27mM. Therefore, it can be said that A2 was capable to show resistance till the Chromium concentration of 27mM. However, from 28mM concentration, no growth of A2 was observed. So, the Minimum Inhibitory Concentration for the A2 isolate is 28mM.

3.1.3 Minimum Inhibitory Concentration of Chromium to inhibit the growth of B1 bacteria

The Minimum Inhibitory Concentration of the isolated sample B1 was determined and the obtained result is given below in the following table.

Table 9: Minimum inhibitory concentration of isolated bacteria B1 against different chromium concentration

Concentration (mM)	Test tube 1	Test tube 2	Test tube 3	Average	Standard deviation
3	0.072	0.074	0.073	0.073	0.001
7	0.07	0.067	0.071	0.069333	0.002081666
10	0.064	0.068	0.057	0.063	0.005567764
15	0.041	0.046	0.047	0.044667	0.00321455
19	0.03	0.03	0.031	0.030333	0.00057735
23	0.018	0.013	0.017	0.016	0.002645751
27	0.004	0.008	0.005	0.005667	0.002081666
30	0	0	0.002	0.000667	0.001154701
31	0	0	0	0	0

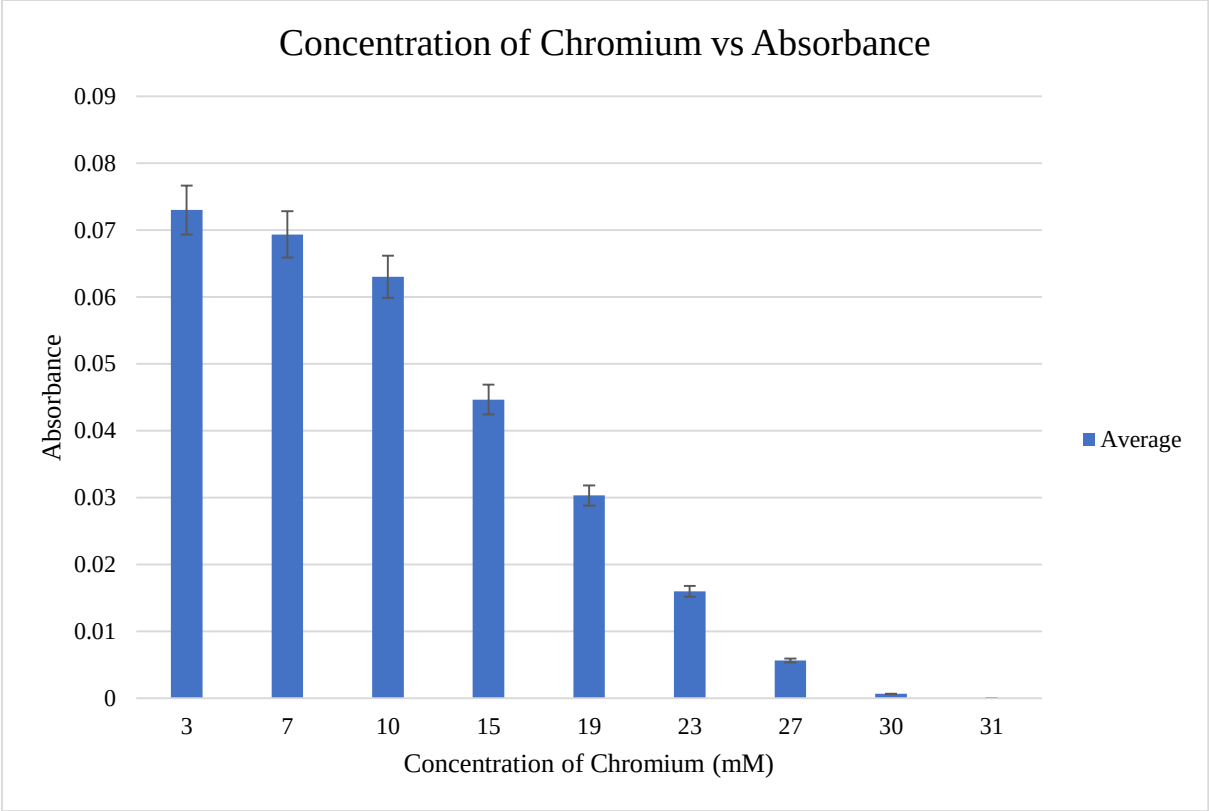

Figure 8: Minimum inhibitory concentration of the isolated sample B1

From the figure, it can be seen that the isolated bacterial strain B1 has the ability to withstand Chromium concentration up to 30mM. Therefore, it can be said that B1 was capable to show resistance till the Chromium concentration of 30mM. However, from 31mM concentration, no growth of B1 was observed. So, the Minimum Inhibitory Concentration for the B1 isolate is 31mM.

3.1.4 Minimum Inhibitory Concentration of Chromium to inhibit the growth of B2 bacteria

The Minimum Inhibitory Concentration of the isolated sample B2 was determined and the obtained result is given below in the following table.

Table 10: Minimum inhibitory concentration of isolated bacteria B2 against different chromium concentration

Concentration (mM)	Test tube 1	Test tube 2	Test tube 3	Average	Standard deviation
3	0.556	0.589	0.531	0.558667	0.029091809
7	0.261	0.273	0.292	0.275333	0.015631165
10	0.112	0.101	0.133	0.115333	0.016258331
15	0.085	0.079	0.088	0.084	0.004582576
19	0.032	0.037	0.04	0.036333	0.004041452
23	0.008	0.008	0.009	0.008333	0.00057735
27	0.002	0.004	0.002	0.002667	0.001154701
28	0	0	0	0	0

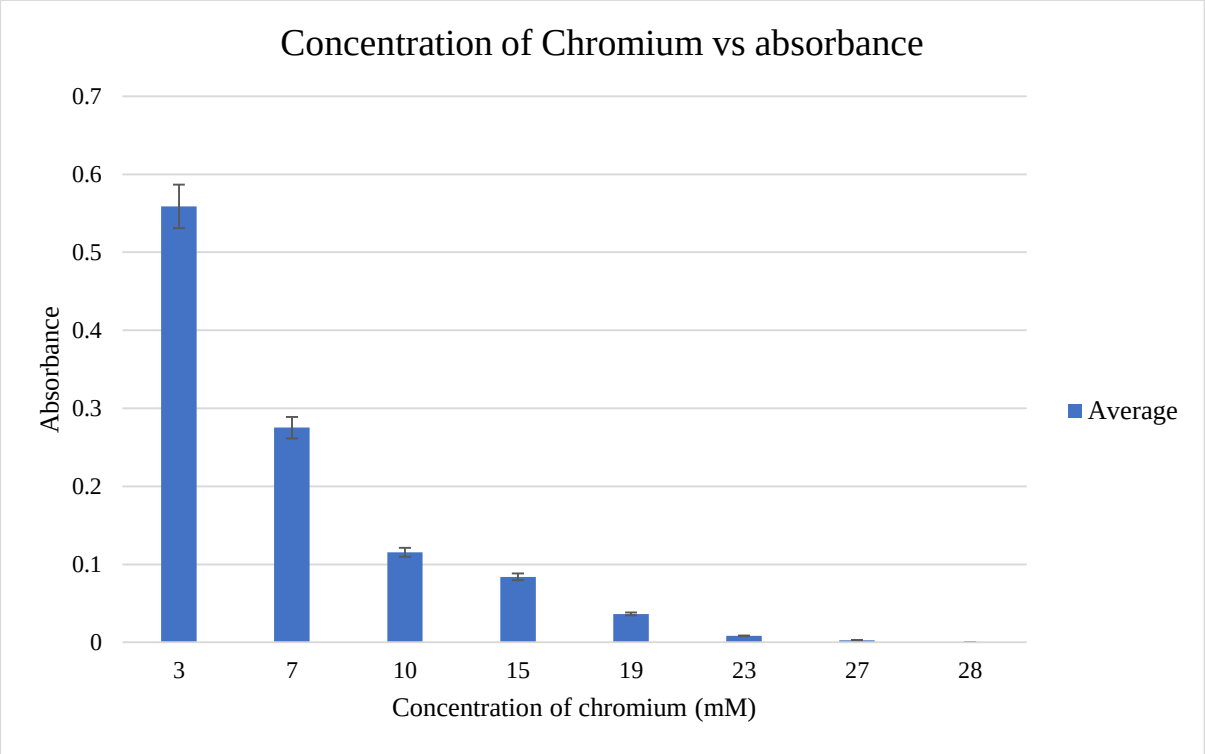

Figure 9: Minimum inhibitory concentration of the isolated sample B2

From the figure, it can be seen that the isolated bacterial strain B2 has the ability to withstand Chromium concentration up to 27mM. Therefore, it can be said that B2 was capable to show resistance till the Chromium concentration of 27mM. However, from 28mM concentration, no growth of B2 was observed. So, the Minimum Inhibitory Concentration for the B2 isolate is 28mM.

3.1.5 Minimum Inhibitory Concentration of Chromium to inhibit the growth of C1 bacteria

The Minimum Inhibitory Concentration of the isolated sample C1 was determined and the obtained result is given below in the following table.

Table 11: Minimum inhibitory concentration of isolated bacteria C1 against different chromium concentration

Concentration (mM)	Test tube 1	Test tube 2	Test tube 3	Average	Standard deviation
3	0.101	0.153	0.099	0.117667	0.0306159
5	0.083	0.089	0.085	0.085667	0.00305505
7	0.087	0.077	0.076	0.08	0.006082763
10	0.061	0.07	0.062	0.064333	0.004932883
15	0.039	0.039	0.033	0.037	0.003464102
17	0.026	0.018	0.021	0.021667	0.004041452
20	0.009	0.008	0.01	0.009	0.001
23	0.001	0.005	0.005	0.003667	0.002309401
25	0	0.001	0.001	0.000667	0.00057735

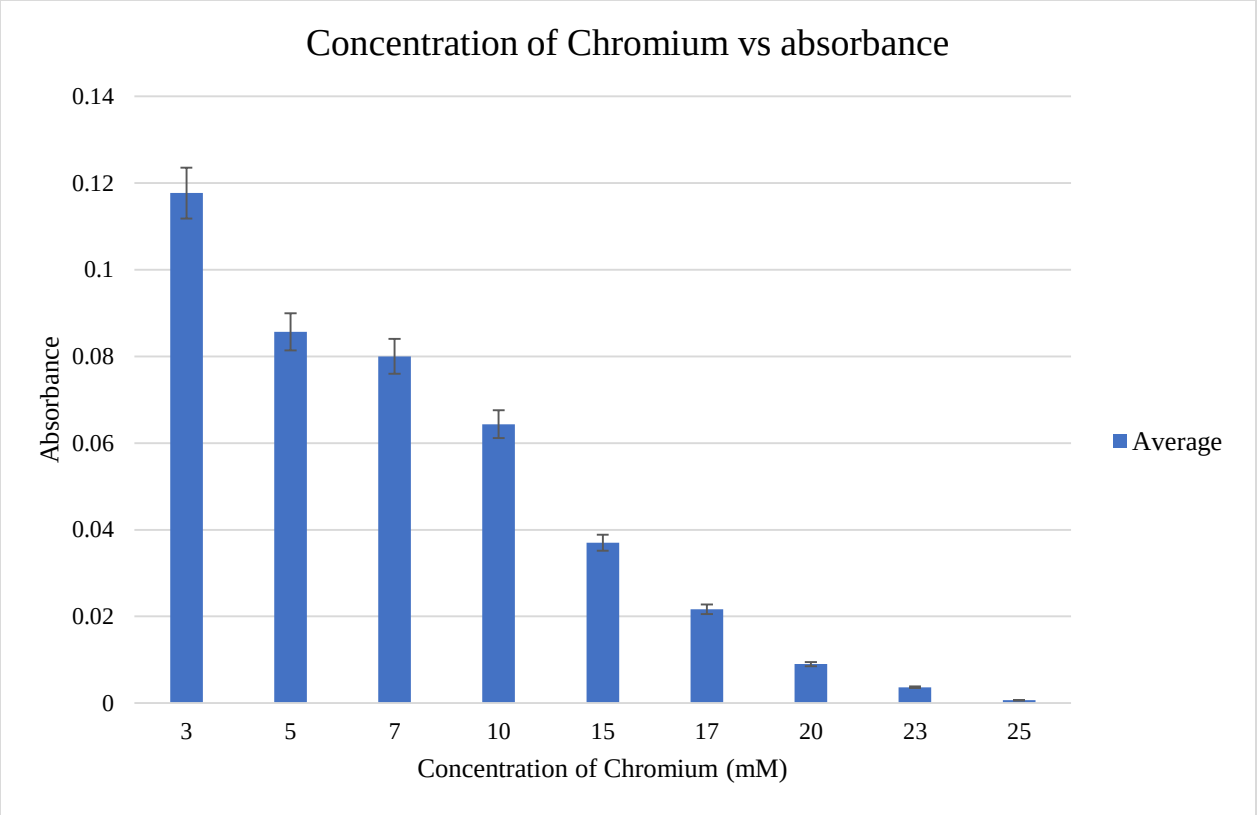

Figure 10: Minimum inhibitory concentration of the isolated sample C1

From the figure, it can be seen that the isolated bacterial strain C1 has the ability to withstand Chromium concentration up to 23mM. Therefore, it can be said that C1 was capable to show resistance till the Chromium concentration of 23mM. However, from 25mM concentration, no growth of C1 was observed. So, the Minimum Inhibitory Concentration for the C1 isolate is 25mM.

3.1.6 Minimum Inhibitory Concentration of Chromium to inhibit the growth of C2 bacteria

The Minimum Inhibitory Concentration of the isolated sample C2 was determined and the obtained result is given below in the following table.

Table 12: Minimum inhibitory concentration of isolated bacteria C2 against different chromium concentration

Concentration (mM)	Test tube 1	Test tube 2	Test tube 3	Average	Standard deviation
3	0.066	0.065	0.066	0.065667	0.00057735
7	0.05	0.058	0.057	0.055	0.004358899
10	0.03	0.028	0.024	0.027333	0.00305505
13	0.011	0.017	0.018	0.015333	0.003785939
15	0.007	0.01	0.008	0.008333	0.001527525
20	0.004	0.001	0.001	0.002	0.001732051
21	0	0	0	0	0

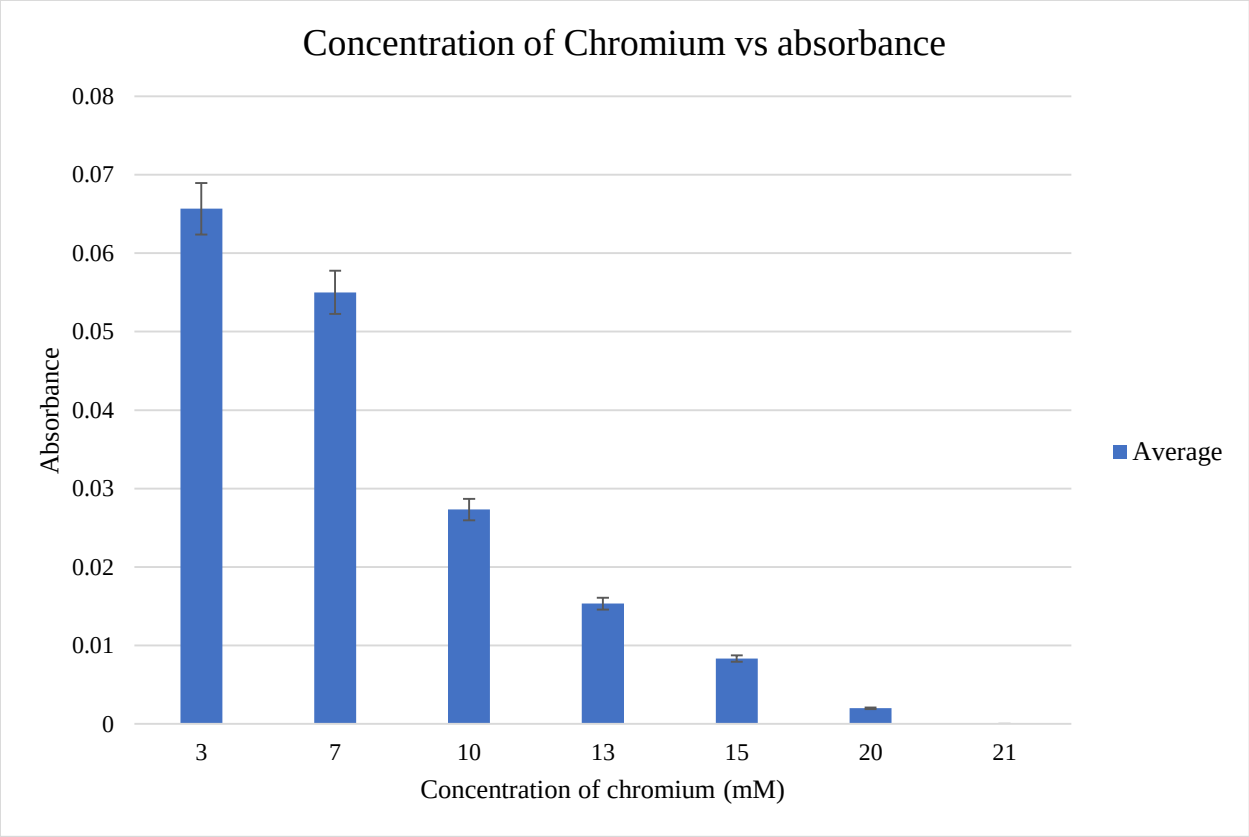

Figure 11: Minimum inhibitory concentration of the isolated sample C2

From the figure, it can be seen that the isolated bacterial strain C2 has the ability to withstand Chromium concentration up to 20mM. Therefore, it can be said that C2 was capable to show resistance till the Chromium concentration of 20mM. However, from 21mM concentration, no growth of C2 was observed. So, the Minimum Inhibitory Concentration for the C2 isolate is 21mM.

3.1.7 Minimum Inhibitory Concentration of Chromium to inhibit the growth of D1 bacteria

The Minimum Inhibitory Concentration of the isolated sample D1 was determined and the obtained result is given below in the following table.

Table 13: Minimum inhibitory concentration of isolated bacteria D1 against different chromium concentration

Concentration (mM)	Test tube 1	Test tube 2	Test tube 3	Average	Standard deviation
3	0.195	0.195	0.198	0.196	0.001732051
5	0.193	0.192	0.197	0.194	0.002645751
7	0.187	0.184	0.184	0.185	0.001732051
10	0.163	0.166	0.17	0.166333	0.003511885
13	0.133	0.149	0.145	0.142333	0.008326664
15	0.131	0.126	0.122	0.126333	0.00450925
19	0.117	0.109	0.115	0.113667	0.004163332
21	0.091	0.099	0.093	0.094333	0.004163332
25	0.069	0.077	0.06	0.068667	0.008504901
30	0.025	0.028	0.023	0.025333	0.002516611
33	0.009	0.001	0.005	0.005	0.004
34	0	0	0	0	0

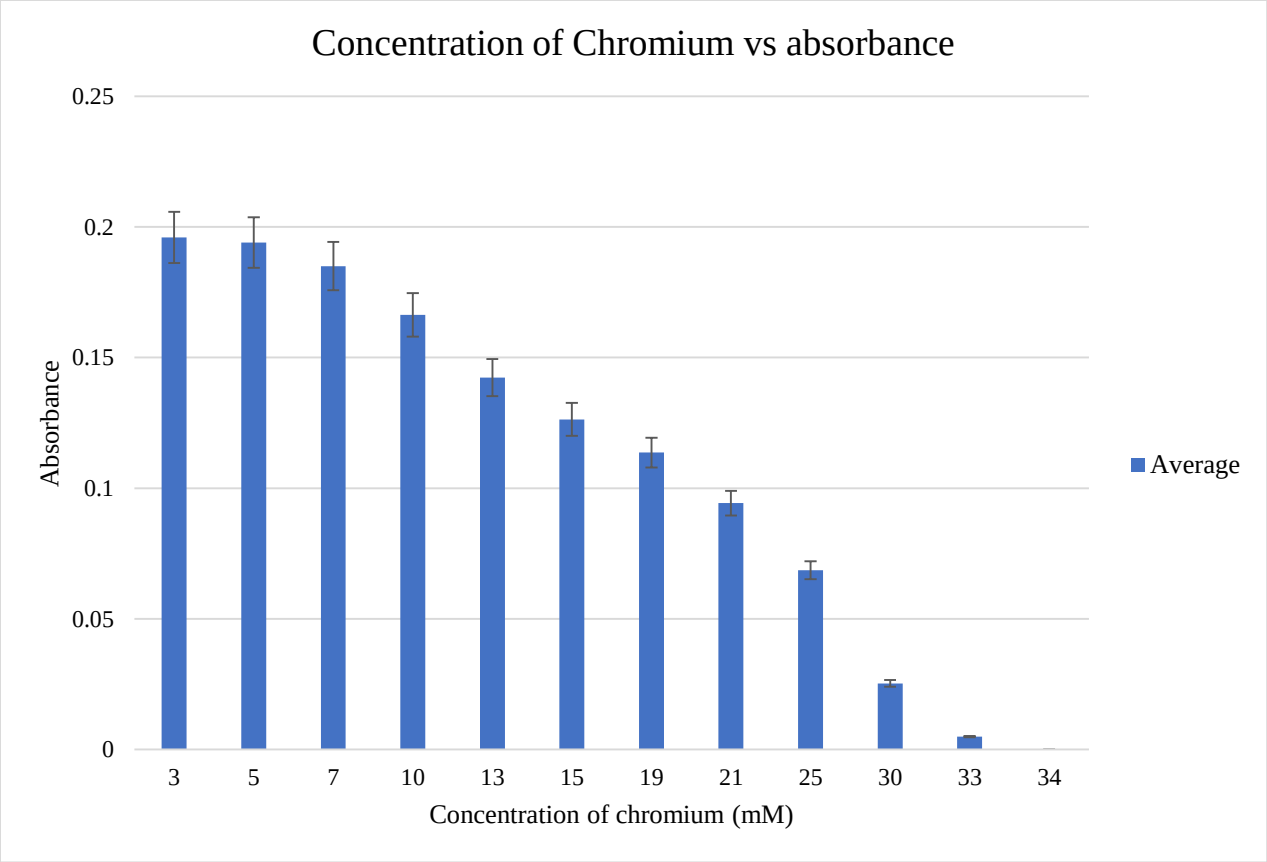

Figure 12: Minimum inhibitory concentration of the isolated sample D1

From the figure, it can be seen that the isolated bacterial strain D1 has the ability to withstand Chromium concentration up to 33mM. Therefore, it can be said that D1 was capable to show resistance till the Chromium concentration of 33mM. However, from 34mM concentration, no growth of D1 was observed. So, the Minimum Inhibitory Concentration for the D1 isolate is 34mM.

3.1.8 Minimum Inhibitory Concentration of Chromium to inhibit the growth of D2 bacteria

The Minimum Inhibitory Concentration of the isolated sample D2 was determined and the obtained result is given below in the following table.

Table 14: Minimum inhibitory concentration of isolated bacteria D2 against different chromium concentration

Concentration (mM)	Test tube 1	Test tube 2	Test tube 3	Average	Standard deviation
3	0.091	0.057	0.099	0.082333	0.022300972
7	0.097	0.075	0.079	0.083667	0.011718931
10	0.061	0.064	0.062	0.062333	0.001527525
13	0.071	0.074	0.06	0.068333	0.007371115
15	0.044	0.049	0.031	0.041333	0.009291573
17	0.043	0.036	0.031	0.036667	0.006027714
20	0.027	0.029	0.021	0.025667	0.004163332
23	0.011	0.009	0.005	0.008333	0.00305505
25	0.001	0.003	0.002	0.002	0.001
26	0	0	0	0	0

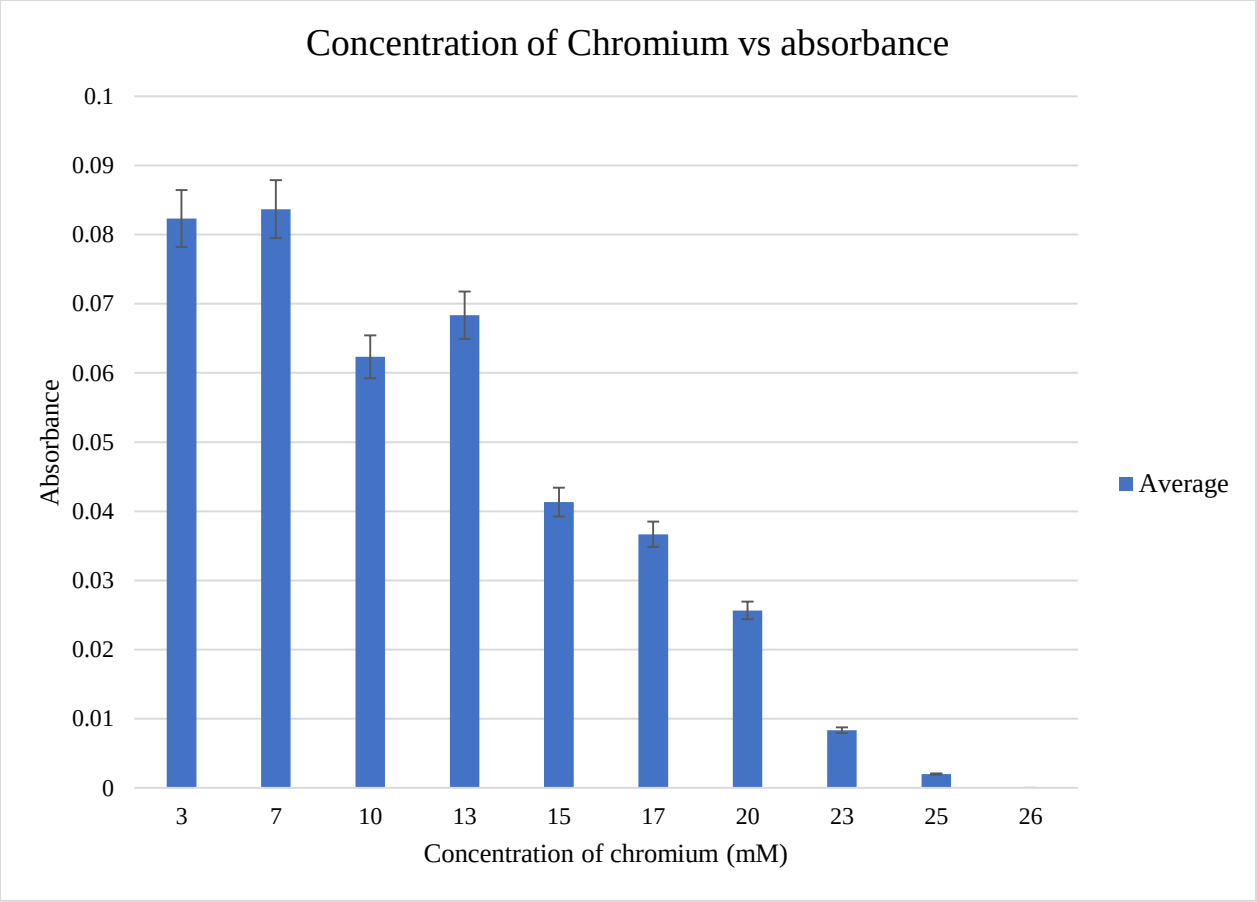

Figure 13: Minimum inhibitory concentration of the isolated sample D2

From the figure, it can be seen that the isolated bacterial strain D2 has the ability to withstand Chromium concentration up to 25mM. Therefore, it can be said that D2 was capable to show resistance till the Chromium concentration of 25mM. However, from 26mM concentration, no growth of D2 was observed. So, the Minimum Inhibitory Concentration for the D2 isolate is 26mM.

3.1.9 Minimum Inhibitory Concentration of Chromium to inhibit the growth of E1 bacteria

The Minimum Inhibitory Concentration of the isolated sample E1 was determined and the obtained result is given below in the following table.

Table 15: Minimum inhibitory concentration of isolated bacteria E1 against different chromium concentration

Concentration (mM)	Test tube 1	Test tube 2	Test tube 3	Average	Standard deviation
3	0.168	0.169	0.171	0.169333	0.001527525
7	0.14	0.137	0.136	0.137667	0.002081666
10	0.136	0.134	0.135	0.135	0.001
13	0.133	0.131	0.13	0.131333	0.001527525
15	0.13	0.125	0.121	0.125333	0.00450925
17	0.109	0.12	0.114	0.114333	0.005507571
20	0.087	0.099	0.081	0.089	0.009165151
23	0.061	0.055	0.05	0.055333	0.005507571
25	0.03	0.03	0.031	0.030333	0.00057735
27	0.017	0.012	0.008	0.012333	0.00450925
30	0.003	0.001	0	0.001333	0.001527525
31	0	0	0	0	0

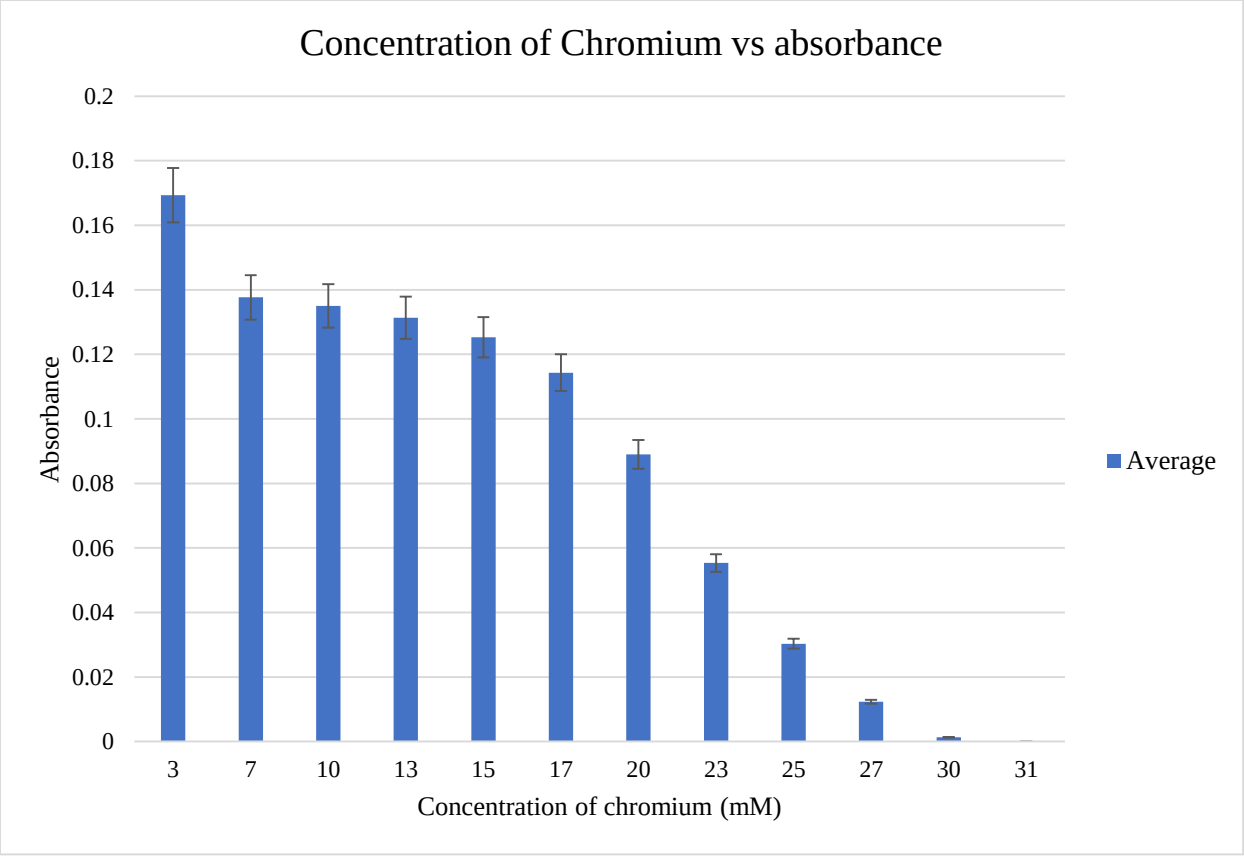

Figure 14: Minimum inhibitory concentration of the isolated sample E1

From the figure, it can be seen that the isolated bacterial strain E1 has the ability to withstand Chromium concentration up to 30mM. Therefore, it can be said that E1 was capable to show resistance till the Chromium concentration of 30mM. However, from 31mM concentration, no growth of E1 was observed. So, the Minimum Inhibitory Concentration for the E1 isolate is 31mM.

3.1.10 Minimum Inhibitory Concentration of Chromium to inhibit the growth of E2 bacteria

The Minimum Inhibitory Concentration of the isolated sample E2 was determined and the obtained result is given below in the following table.

Table 16: Minimum inhibitory concentration of isolated bacteria E2 against different chromium concentration

Concentration (mM)	Test tube 1	Test tube 2	Test tube 3	Average	Standard deviation
3	0.009	0.011	0.017	0.01233333	0.004163332
7	0.007	0.005	0.007	0.00633333	0.001154701
10	0.008	0.004	0.003	0.005	0.002645751
13	0.001	0.001	0.002	0.00133333	0.001333333
15	0	0	0	0	0

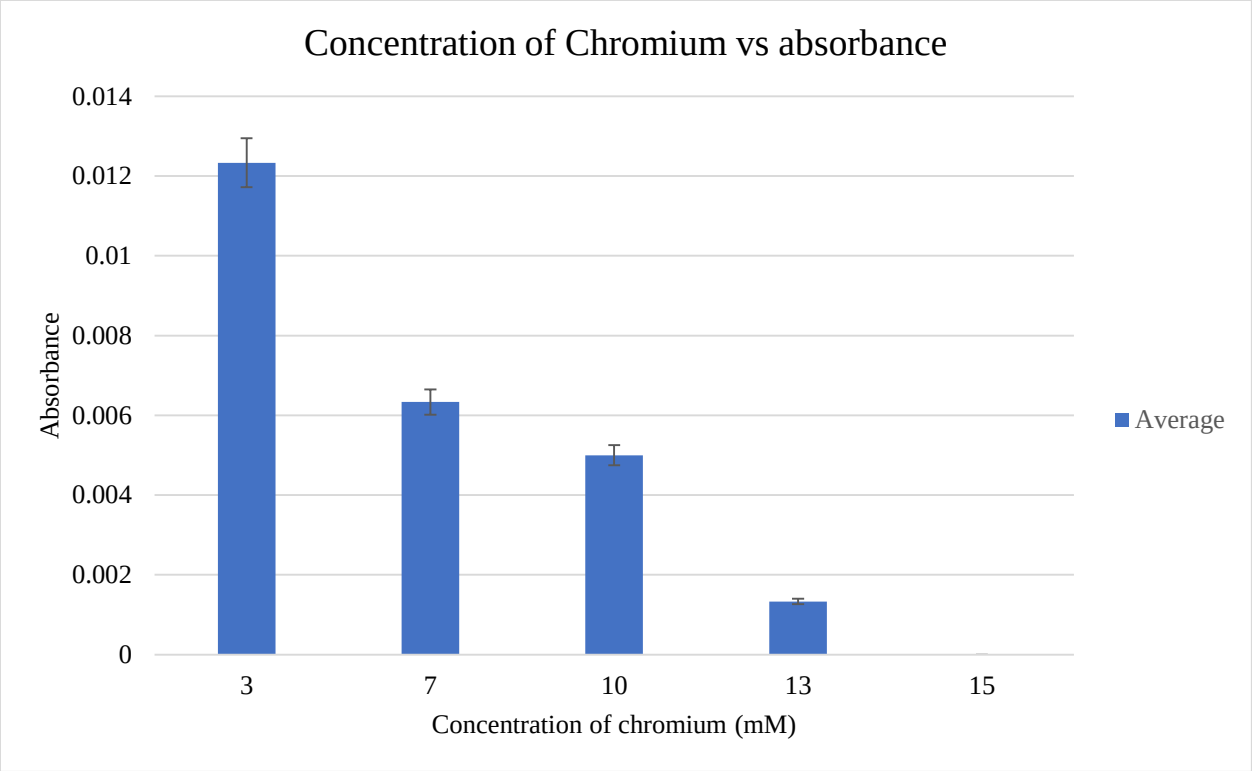

Figure 15: Minimum inhibitory concentration of the isolated sample E2

From the figure, it can be seen that the isolated bacterial strain E2 has the ability to withstand Chromium concentration up to 13mM. Therefore, it can be said that E2 was capable to show resistance till the Chromium concentration of 13mM. However, from 15mM concentration, no growth of E2 was observed. So, the Minimum Inhibitory Concentration for the E2 isolate is 15mM.

3.1.11 Minimum Inhibitory Concentration of Chromium to inhibit the growth of F1 bacteria

The Minimum Inhibitory Concentration of the isolated sample F1 was determined and the obtained result is given below in the following table.

Table 17: Minimum inhibitory concentration of isolated bacteria F1 against different chromium concentration

Concentration (mM)	Test tube 1	Test tube 2	Test tube 3	Average	Standard deviation
3	0.014	0.014	0.016	0.0147	0.001154701
7	0.012	0.011	0.012	0.0117	0.00057735
10	0.010	0.009	0.011	0.01	0.001
13	0.007	0.008	0.006	0.007	0.001
15	0.003	0.003	0.001	0.0023	0.001154701
16	0	0	0	0	0

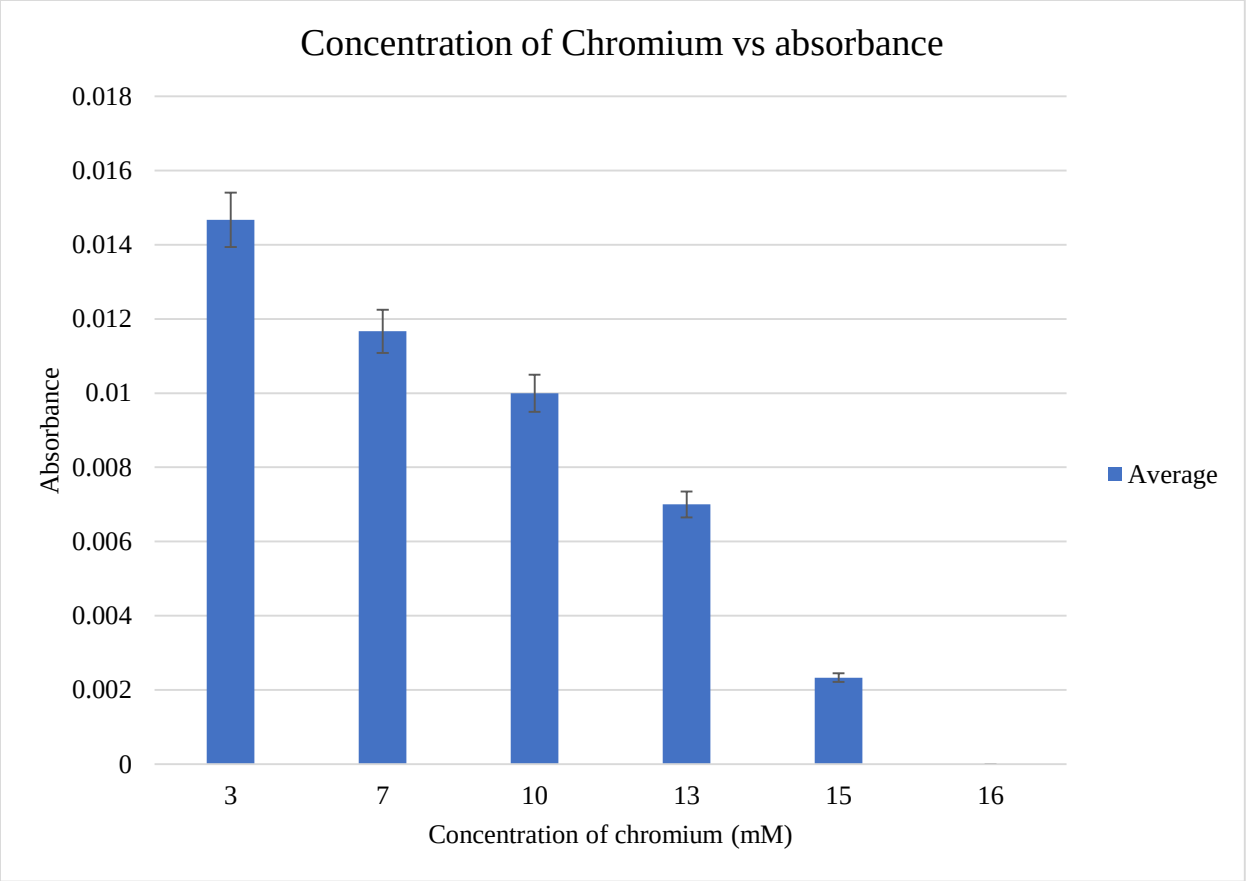

Figure 16: Minimum inhibitory concentration of the isolated sample F1

From the figure, it can be seen that the isolated bacterial strain F1 has the ability to withstand Chromium concentration up to 15mM. Therefore, it can be said that F1 was capable to show resistance till the Chromium concentration of 15mM. However, from 16mM concentration, no growth of F1 was observed. So, the Minimum Inhibitory Concentration for the F1 isolate is 16mM.

3.1.12 Minimum Inhibitory Concentration of Chromium to inhibit the growth of F2 bacteria

The Minimum Inhibitory Concentration of the isolated sample F2 was determined and the obtained result is given below in the following table.

Table 18 Minimum inhibitory concentration of isolated bacteria F2 against different chromium concentration

Concentration (mM)	Test tube 1	Test tube 2	Test tube 3	Average	Standard deviation
3	0.038	0.033	0.039	0.0366667	0.00321455
7	0.029	0.027	0.022	0.026	0.003605551
10	0.021	0.025	0.022	0.0226667	0.002081666
15	0.013	0.014	0.015	0.014	0.001
20	0.005	0.005	0.006	0.0053333	0.00057735
21	0	0	0	0	0

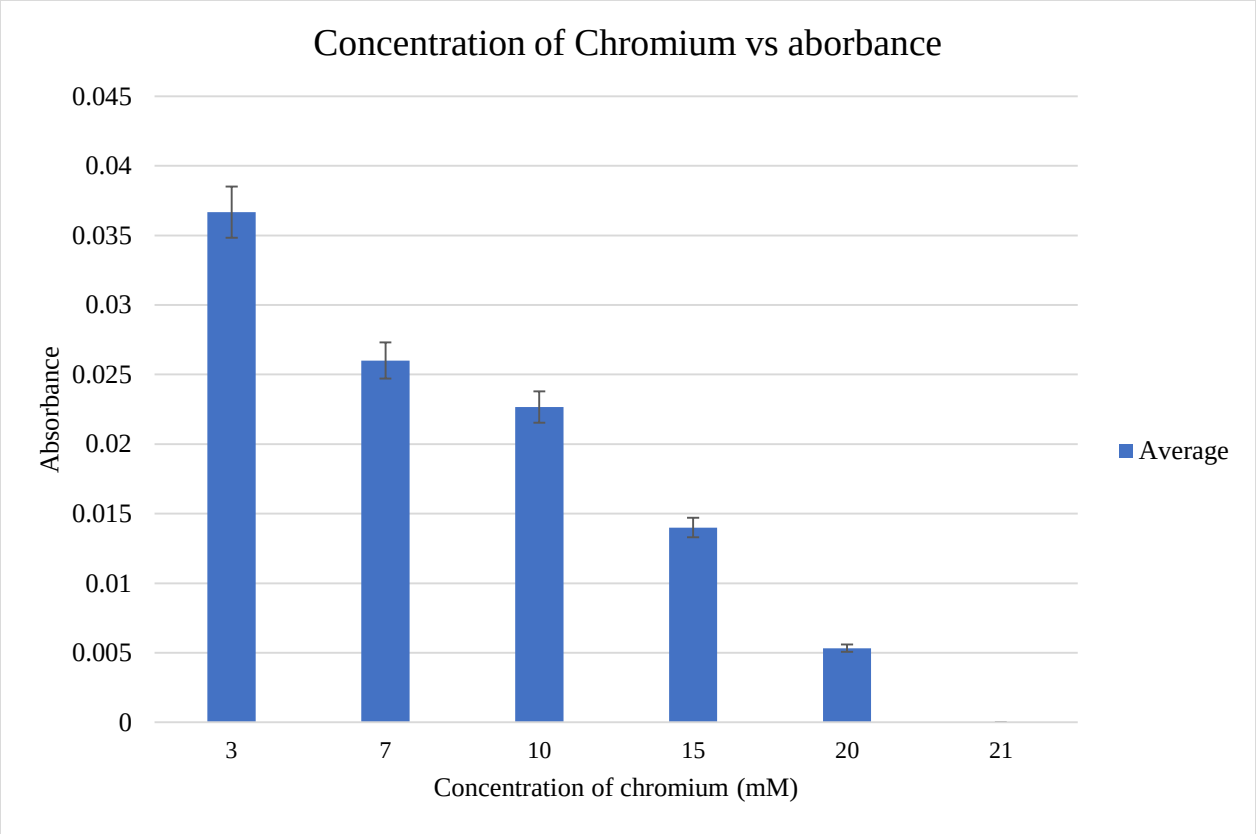

Figure 17: Minimum inhibitory concentration of the isolated sample F2

From the figure, it can be seen that the isolated bacterial strain F2 has the ability to withstand Chromium concentration up to 20mM. Therefore, it can be said that F2 was capable to show resistance till the Chromium concentration of 20mM. However, from 21mM concentration, no growth of F2 was observed. So, the Minimum Inhibitory Concentration for the F2 isolate is 21mM.

3.1.13 Minimum Inhibitory Concentration of Chromium to inhibit the growth of G1 bacteria

The Minimum Inhibitory Concentration of the isolated sample G1 was determined and the obtained result is given below in the following table.

Table 19: Minimum inhibitory concentration of isolated bacteria G1 against different chromium concentration

Concentration (mM)	Test tube 1	Test tube 2	Test tube 3	Average	Standard deviation
3	0.981	0.99	1.001	0.990667	0.010016653
5	0.898	0.901	0.91	0.903	0.006244998
7	0.77	0.715	0.756	0.747	0.028583212
10	0.506	0.517	0.509	0.510667	0.005686241
13	0.317	0.311	0.322	0.316667	0.005507571
15	0.199	0.216	0.192	0.202333	0.012342339
17	0.1	0.099	0.098	0.099	0.001
20	0.069	0.068	0.076	0.071	0.004358899
25	0.021	0.028	0.017	0.022	0.005567764
27	0.011	0.008	0.007	0.008667	0.002081666
30	0.002	0.003	0.003	0.002667	0.00057735
31	0	0	0	0	0

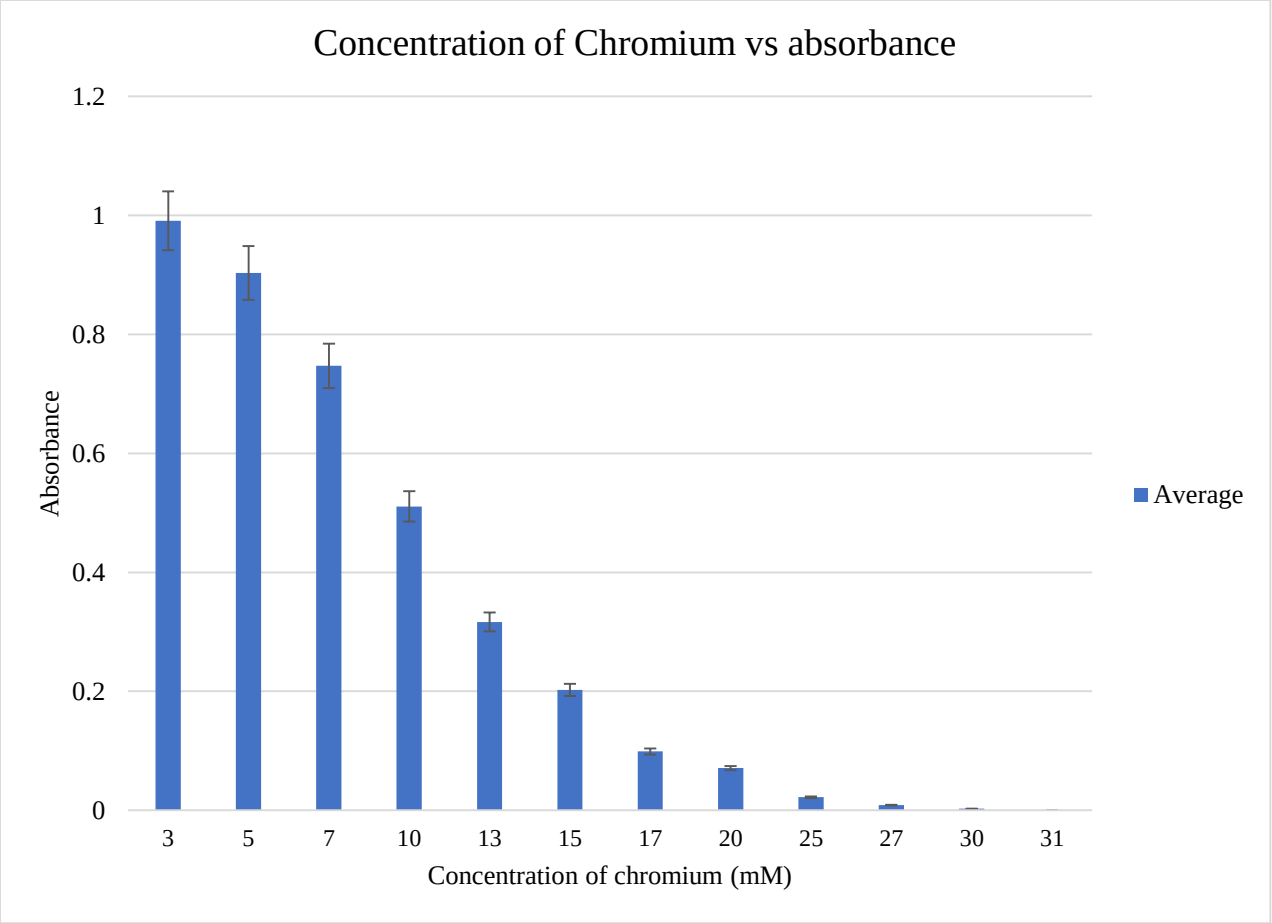

Figure 18: Minimum inhibitory concentration of the isolated sample G1

From the figure, it can be seen that the isolated bacterial strain G1 has the ability to withstand Chromium concentration up to 30mM. Therefore, it can be said that G1 was capable to show resistance till the Chromium concentration of 30mM. However, from 31mM chromium concentration, no growth of G1 was observed. So, the Minimum Inhibitory Concentration for the G1 isolate is 31mM.

3.1.14 Minimum Inhibitory Concentration of Chromium to inhibit the growth of G2 bacteria

The Minimum Inhibitory Concentration of the isolated sample G2 was determined and the obtained result is given below in the following table.

Table 20: Minimum inhibitory concentration of isolated bacteria G2 against different chromium concentration

Concentration (mM)	Test tube 1	Test tube 2	Test tube 3	Average	Standard deviation
3	1.997	1.999	1.999	1.998333	0.001154701
5	1.908	1.911	1.951	1.923333	0.024006943
10	1.856	1.877	1.852	1.861667	0.013428825
15	1.66	1.681	1.658	1.666333	0.01274101
19	1.378	1.329	1.38	1.362333	0.028884829
23	1.11	1.109	1.099	1.106	0.006082763
25	1.005	1.097	1.085	1.062333	0.050013332
27	1.068	1.051	1.063	1.060667	0.008736895
30	0.995	0.987	0.912	0.964667	0.045785733
33	0.814	0.755	0.713	0.760667	0.050737889
35	0.4	0.337	0.374	0.370333	0.031659648
37	0.29	0.199	0.216	0.235	0.048383882
40	0.077	0.089	0.081	0.082333	0.006110101
42	0.043	0.028	0.02	0.030333	0.011676187
44	0.007	0.003	0.006	0.005333	0.002081666
45	0	0	0	0	0

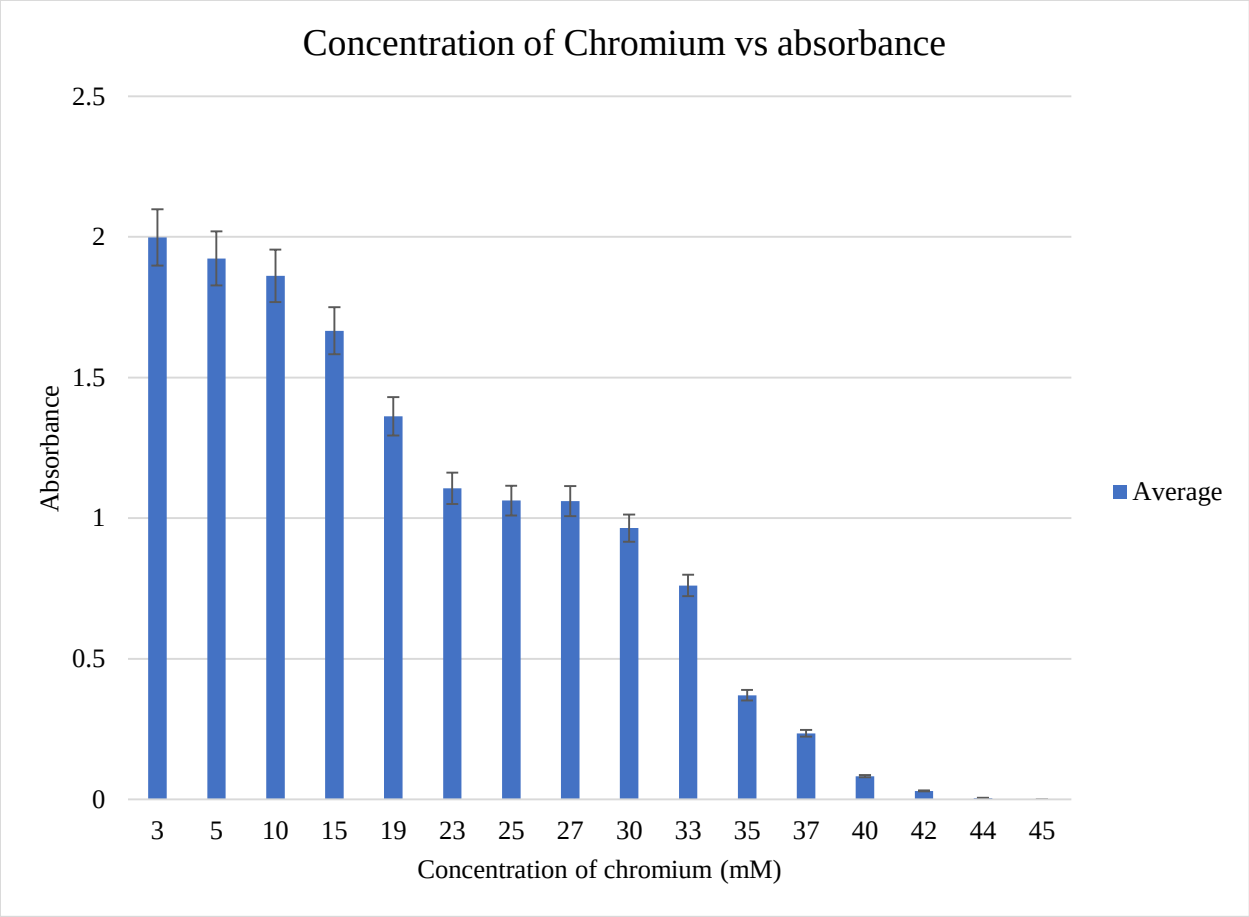

Figure 19: Minimum inhibitory concentration of the isolated sample G2

From the figure, it can be seen that the isolated bacterial strain G2 has the ability to withstand Chromium concentration up to 44mM. Therefore, it can be said that G2 was capable to show resistance till the Chromium concentration of 44mM. However, from 45mM chromium concentration, no growth of G2 was observed. So, the Minimum Inhibitory Concentration for the G2 isolate is 45mM.

3.2 Result summary

The given table summarizes the result of the minimum inhibitory concentration test of chromium again 14 bacterial strains.

Table 21: MIC of chromium to different bacterial strains

Name of the bacterial strain	MIC of Chromium
A1	24mM
A2	28mM
B1	31mM
B2	28mM
C1	25mM
C2	21mM
D1	34mM
D2	26mM
E1	31mM
E2	15mM
F1	16mM
F2	21mM
G1	31mM
G2	45mM

3.3 Discussion

Chromium is abundant in nature and spread out in the environment. In Bangladesh, existence of Chromium is from the dyeing and tannery industries. At present, the amount of chromium present in environment has become life-threatening to humankind as well as to all living beings. But as the carcinogenic property of chromium is now exposed to human, it has now become one of the greatest concerns of people to find out the proper and valid solution for removal of not only chromium but also all the toxic heavy metals. We preferred bioremediation over other processes for our study. The purpose of this study was to find out the minimum inhibitory concentration of Chromium to different bacterial strains that were collected from the environment.

From the results, it was seen that each of the bacteria could withstand chromium to different concentration. After the concentration of the samples was found to be 0 in each case in the UV spectroscopy. This signifies, after the concentration no bacterial growth can occur in the chromium-based environment. The concentration after which no bacterial growth was found was the minimum concentration for our experiment. For bacterial strain A1 the minimum inhibitory concentration for A1 strain was 24mM. Again, the bacterial strains A2 the minimum inhibitory concentration was 28mM. For bacterial strains B1 the minimum inhibitory concentration for B1 bacterial strain was 31mM. Also, the minimum inhibitory concentration for B2 bacterial strain was 28mM. Moreover, the minimum inhibitory concentration for C1 bacterial strain was 25mM. For bacterial strain C2, the minimum inhibitory concentration was 21mM. Furthermore, the minimum inhibitory concentration for D1 bacterial strain was 34mM. Again, the minimum inhibitory concentration for D2 bacterial strain was found 26mM. Bacterial strain E1 could withstand the concentration of Chromium up to 30mM. the minimum inhibitory concentration for E1 bacterial strain was 31mM. The minimum inhibitory concentration for E2 bacterial strain was 16mM. The

minimum inhibitory concentration for F1 bacterial strain was 16mM. Again, it was seen bacterial strains F2 were able to withstand the concentration of Chromium up to 20mM. The minimum inhibitory concentration for F2 bacterial strain was 21mM. Minimum inhibitory concentration of bacterial strains G1 was 31mM. Bacterial strain G2 showed the minimum inhibitory concentration of 45mM.

Therefore, the highest minimum inhibitory concentration of chromium was against G2 bacterial strain (45mM). So, G2 the most tolerable bacterial strain among the bacteria to heavy metal chromium. Again, the lowest minimum inhibitory concentration of chromium was against E2 bacterial strain (15mM). So, E2 was the least tolerable to chromium. Most of the bacteria showed resistance to chromium concentration above 20mM. Some of the bacterial strains were also resistance to chromium concentration above 30mM.

Chapter 4

Conclusion and future works

4.1 Conclusion

To conclude with, in the experiment, some of the bacterial strain carries ability to resist high concentration of chromium. Specially, G2 and D1 bacterial strain can tolerate chromium concentration up to 45mM and 34mM consecutively. Also, few bacterial strains can withstand chromium above 30mM. In favorable condition, the bacteria may tolerate chromium at higher concentration. The capability of the strains demonstrates their defense mechanism against chromium. So, these bacteria have biologically chromium reducing properties. These properties play a significant role in bioremediation.

4.2 Future works

After testing the minimum inhibitory concentration, further studies on bacteria and heavy metals can be done. The bacterial mechanism of reducing chromium can be studied. Also, identification of characteristics of each bacterial strain can be done. Moreover, plasmid analysis can be done to find out more about the bacteria.

References

Achmad, R. T., Budiawan, & Auerkari, E. I. (2017). Effects of chromium on human body. *Annual Research and Review in Biology*, *13*(2), 1–8. https://doi.org/10.9734/ARRB/2017/33462

Ackerley, D. F., Gonzalez, C. F., Park, C. H., Balke II, R., Keyhan, M., & Matin, A. (2003). Chromate-Reducing Properties of Soluble Flavoproteins from Pseudomonas putida and Escherichia coli D. *APPLIED AND ENVIRONMENTAL MICROBIOLOGY*, *70*(2), 873–882. https://doi.org/10.1128/AEM.70.2.873

Ahammed, S. S., Tasfina, S., Rabbani, K. A., & Khaleque, A. (2015). An Investigation Into The Water Quality Of Buriganga - A River Running Through Dhaka. *International Journal of Scientific & Technology Research*, *4*(8), 36–41.

Andrews, J. M. (2001). Determination of minimum inhibitory concentration. *Journal of Antimicrobial Chemotherapy*, *48*(1), 5–16.

Asomugha, R. N., Udowelle, N. A., Offor, S. J., Njoku, C. J., Ofoma, I. V., Chukwuogor, C. C., & Orisakwe, O. E. (2016). Heavy metals hazards from Nigerian spices. *Roczniki Panstwowego Zakladu Higieny*, *67*(3), 309–314.

Atiqur Raman Sunny , S. Naznin, M. J. Rahman, M. N. and M. A. W. (2017). *Assessment of the river water quality parameters and pollution: An insight from Dhaka city SEE PROFILE*. 23–24. Retrieved from https://www.researchgate.net/publication/319737220

Awokunmi, E. E., Asaolu, S. S., Adefemi, S. O., & Gbolagade, A. Y. (2015). Contributions of Municipal Solid Waste to Heavy Metal Concentration in Soil Near Oke Ese Dumpsite, Ilesha, Osun State, Nigeria. *International Journal of Environmental Protection*, *5*(1), 44–51.

https://doi.org/10.5963/ijep0501007

Cheng, Y., Holman, H. Y., & Lin, Z. (2012). Remediation of chromium and uranium contamination by microbial activity. *Elements*, *8*(2), 107–112. https://doi.org/10.2113/gselements.8.2.107

DesMarias, T. L., & Costa, M. (2019). Mechanisms of chromium-induced toxicity. *Current Opinion in Toxicology*, *14*(Iii), 1–7. https://doi.org/10.1016/j.cotox.2019.05.003

Drahansky, M., Paridah, M. ., Moradbak, A., Mohamed, A. ., Owolabi, F. abdulwahab taiwo, Asniza, M., & Abdul Khalid, S. H. . (2016). Mechanism and Health Effects of Heavy Metal Toxicity in Humans. In *Intech* (Vol. 1). https://doi.org/http://dx.doi.org/10.5772/57353

Duruibe, J. O., Ogwuegbu, M. O. C. and Egwurugwu, J. N. (2007). Heavy metal pollution and human biotoxic effects. *International Journal of Physical Sciences*, *2*(5), 112–118.

Gad, S. C. (1989). Acute and chronic systemic chromium toxicity. *Science of the Total Environment, The*, *86*(1–2), 149–157. https://doi.org/10.1016/0048-9697(89)90201-5

Gorchev, H. G., & Ozolins, G. (2004). Guidelines for Drinking-water Quality, 3rd Edition. *World Health Organization*, *1*, 564. https://doi.org/10.1016/S1462-0758(00)00006-6

Guo, H., Luo, S., Chen, L., Xiao, X., Xi, Q., Wei, W., … He, Y. (2010). Bioremediation of heavy metals by growing hyperaccumulaor endophytic bacterium Bacillus sp. L14. *Bioresource Technology*, *101*(22), 8599–8605. https://doi.org/10.1016/j.biortech.2010.06.085

Ianeva, O. D. (2009). Mechanisms of bacteria resistance to heavy metals. *Mikrobiolohichnyĭ Zhurnal (Kiev, Ukraine : 1993)*, *71*(6), 54–65.

Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B. B., & Beeregowda, K. N. (2014). Toxicity,

mechanism and health effects of some heavy metals. *Interdisciplinary Toxicology*, *7*(2), 60–72. https://doi.org/10.2478/intox-2014-0009

Järup, L. (2003). Hazards of heavy metal contamination. *British Medical Bulletin*, *68*, 167–182. https://doi.org/10.1093/bmb/ldg032

Kibria, M. G. . (2015). *Buriganga River Pollution : Its Causes and Impacts Paper ID : EE-052*. (December).

Mahmood, S., Nourin, F. T. J., Siddika, A., & Khan, T. F. (2017). Encroachment of the Buriganga River in Bangladesh. *Journal of Minerals and Materials Characterization and Engineering*, *05*(05), 266–273. https://doi.org/10.4236/jmmce.2017.55022

Malik, D., Singh, S., Thakur, J., Kaur, A., & Nijhawan, S. (2016). A Review on oxidative burst : Biochemical implications of metal toxicity A Review on oxidative burst : Biochemical implications of metal toxicity. *Journal of Biotechnology and Crop Science*, *5*(7), 35–47.

Oliveira, H. (2012). Chromium as an Environmental Pollutant: Insights on Induced Plant Toxicity. *Journal of Botany*, *2012*, 1–8. https://doi.org/10.1155/2012/375843

Paul, D. (2017). Research on heavy metal pollution of river Ganga: A review. *Annals of Agrarian Science*, *15*(2), 278–286. https://doi.org/10.1016/j.aasci.2017.04.001

Polti, M. A., García, R. O., Amoroso, M. J., & Abate, C. M. (2009). Bioremediation of chromium(VI) contaminated soil by Streptomyces sp. MC1. *Journal of Basic Microbiology*, *49*(3), 285–292. https://doi.org/10.1002/jobm.200800239

Sarkar, M., Rahman, A. L., Islam, J., Ahmed, K., Uddin, M., & Bhoumik, N. (2015). Study of hydrochemistry and pollution status of the Buriganga river, Bangladesh. *Bangladesh Journal*

of Scientific and Industrial Research, *50*(2), 123–134. https://doi.org/10.3329/bjsir.v50i2.24353

Sarwar, N., Imran, M., Shaheen, M. R., Ishaque, W., Kamran, M. A., Matloob, A., … Hussain, S. (2017). Phytoremediation strategies for soils contaminated with heavy metals: Modifications and future perspectives. *Chemosphere*, *171*(April 2018), 710–721. https://doi.org/10.1016/j.chemosphere.2016.12.116

Sellamuthu, R., Umbright, C., Chapman, R., Leonard, S., & Li, S. (2011). Transcriptomics Evaluation of Hexavalent Chromium Toxicity in Human Dermal Fibroblasts. *Journal of Carcinogenesis & Mutagenesis*, *02*(01), 1–8. https://doi.org/10.4172/2157-2518.1000116

Sethunathan N, N. R. K. S. M. M. J. AL. (2003). Chromium-microorganism interactions in soils: Remediation implications. *Reviews of Environmental Contamination and Toxicology*, *178*, 93–164. https://doi.org/10.1007/0-387-21728-2

Shanker, A. K., Cervantes, C., Loza-Tavera, H., & Avudainayagam, S. (2005). Chromium toxicity in plants. *Environment International*, *31*(5), 739–753. https://doi.org/10.1016/j.envint.2005.02.003

Stohs, S. J., & Bagchi, D. (1995). Oxidative mechanisms in the toxicity of metal ions. *Free Radical Biology and Medicine*, *18*(2), 321–336. https://doi.org/10.1016/0891-5849(94)00159-H

Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., & Sutton, D. J. (2012). Heavy Metals Toxicity and the Environment. *Molecular, Clinical and Environmental Toxicology*, *101*, 133–164. https://doi.org/10.1007/978-3-7643-8340-4

Torres-Martínez, L. M., Kharissova, O. V., & Kharisov, B. I. (2019). Handbook of ecomaterials.

In *Handbook of Ecomaterials* (Vol. 1). https://doi.org/10.1007/978-3-319-68255-6

Wuana, R. A., & Okieimen, F. E. (2011). Heavy Metals in Contaminated Soils: A Review of Sources, Chemistry, Risks and Best Available Strategies for Remediation. *ISRN Ecology*, *2011*, 1–20. https://doi.org/10.5402/2011/402647

Zahoor, A., & Rehman, A. (2009). Isolation of Cr(VI) reducing bacteria from industrial effuents and their potential use in bioremediation of chromium containing wastewater. *Journal of Environmental Sciences*, *21*, 814–820.

Zhang, X. H., Zhang, X., Wang, X. C., Jin, L. F., Yang, Z. P., Jiang, C. X., … Zhu, Y. M. (2011). Chronic occupational exposure to hexavalent chromium causes DNA damage in electroplating workers. *BMC Public Health*, *11*(1), 224. https://doi.org/10.1186/1471-2458-11-224