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

By

Anika Tabassum Shama

ID: 15346007

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/our 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.

Student's Full Name & Signature:

Anika Tabassum Shama
15346007

Approval

The thesis/project titled “Detrmination of minimum inhibitory concentration of cadmium salts on the microbial strains isolated from Buriganga river-bed soil” submitted by Anika Tabassum Shama (15346007) of Summer, 2015 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.) on October, 2019.

Examining Committee:

_____

Md. Samiul Alam Rajib
Senior lecturer, Department of Pharmacy
Brac University

_____

Dr. Hasina Yasmin
Professor, Department of Pharmacy
Brac University

_____

Prof. Dr. Eva Rahman Kabir
Chairperson, Department of Pharmacy
Brac University

Abstract

Heavy metals are abundantly available in our environment. Most of them are toxic in high concentration. One of those heavy metals is Cadmium which is released from tannery, dye, chemical industries in broad range and spreading toxicity to the environment and to the living organisms. Bioremediation is a process by which it is possible to reduce heavy metals from the environment using micro-organisms. Heavy metal resistant bacterial strains are the best choice of micro-organisms in this bio-remediation process. In this study we have worked with 14 different types of Chromium resistant bacteria and analyzed their resistance capacity towards Cadmium. The purpose of this study was to find multiple heavy metal resistant bacterial strains that can be used to remove heavy metals from environment for the betterment of the human beings as well as for all the other living organisms.

Keywords: heavy metal; cadmium; toxicity; bioremediation; MIC

This work is dedicated to my parents for their continuous love and support.

Acknowledgement

First of all, I am grateful to Almighty Allah for giving me the strength and dedication 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
PAH	Polycyclic Aromatic Hydrocarbons
EPA	Environmental Protection Agency
EPS	Extracellular Polymeric Substance
CDF	Cation diffusion facilitator
RND	Resistance Nodulation cell Division
CDA	Carbamoyl-phosphate synthetase 2, Aspartate transcarbamylase, and Dihydroorotase
PAD	Peripheral artery disease
MI	Myocardial Infarction
AMP	Amplified Musculoskeletal Pain
NA	Nutrient Agar
UV-Vis	Ultra Violet Visible
mM	Milli-molar
mL	Milliliter
µg	Micro-gram
mg	Milligram

Chapter 1

INTRODUCTION

1.1 Background

In present, a dramatic increase of the heavy metals can be observed as a result of the aggressive use of them in agricultural, pharmaceutical, atmospheric, industrial, domestic and technological purpose. Heavy metals are constant in nature and are called environmental pollutant as they are affecting the plants, animals as well as the human beings (Rajeswari, 2014). In high concentration heavy metals are effectively toxic for the living organisms; however some of them can also exhibit their toxic effects in lower concentrations. Heavy metals can originate naturally and from anthropogenic sources. The sources of these metals are: rock weathering, sea-salt sprays, volcanic eruptions, biogenic sources, forest fire and solid air-borne particles. Human activities including Industrial and agricultural works, metallurgical processes, automobile exhaust also causes the release of heavy metals. Composts that containing these metals can affect the physical and chemical properties of soil. Metal uptake by the plants from this soil, inhibits physiological metabolism and thus reduces the production of crops. Heavy metals uptake creates hazards for both the human health and also to the environment (Jiwan & S, 2014).

Heavy metals are the trace elements that can neither be destroyed nor degraded. They are considered as one of the most hazardous and common element which is more than 5 g/cm3 dense. (Yadav, Gupta, Kumar, & Singh, 2017). Some metals are essential for life process, for example copper, iron, manganese and zinc whereas there are some other metals like lead, chromium, cadmium, nickel and mercury which can often spread harmful effects. Metals are harmful because they have a tendency to accumulate. Heavy metals are considered as

systemic toxicant, they have the ability to damage multiple organ even at a low concentration. These heavy metals are classified as human carcinogens (Tchounwou, Yedjou, Patlolla, & Sutton, 2013). Bioremediation is one of the best ways to reduce metals from the environment. It is most preferred to use bioremediation using microbial systems as it can be done at a lower cost and least amount of waste is generated using this technique (Yadav et al., 2017). It is mainly a process which uses microbes or its enzyme to reduce the toxicity of heavy metals and thus removes pollutants from the environment. With the help of the micro-organisms that have multiple heavy metal resistance property it will be easier to incorporate them in the bioremediation process where they can help in removing multiple metals from a specific region.

Therefore, the study is designed to isolate bacterial strains that have multiple metal resistance properties. In this study we are going to analysis the presence and intensity of cadmium resistance properties of the bacterial strains that are already chromium resistance.

1.2 Aim and Objectives:

The hypothesis of this project is to check the heavy metal resistance pattern of some isolated bacterial strains which are already chromium resistant. The objectives of this purpose are:

1. Analyze the pattern of growth of the bacterial strains in Cadmium rich environment.

2. Measurement of the MIC of cadmium of the bacterial strains.

3. Evaluate the MIC of Cadmium to find out the resistant and tolerance level of bacteria.

1.3 Literature Review

1.3.1 Heavy Metal

The term “heavy metal” means a metal or a metalloid that has a density which exceeds 5g /cm−3. Heavy metals are usually associated with toxicity and pollution. However some of these heavy metals are actually consumed by organisms where they are present at lower concentrations (Singh et al., 2018). Heavy metals are of high and specific weights. They have higher density compared to other elements. There are different opinions of different authors regarding the exact density of heavy metals (Duffus, 2002). According to most of the cited references , the approximate density of a heavy metal is from 4.5gmc-3 or 5gcm-3 (Appenroth, 2010).

1.3.2 Examples of Heavy Metals

List of heavy metals are given in Table 1 (Protocol et al., 2010).

Table 1 List of heavy metals

Gold	Bismuth	Cadmium	Cesium	Cerium	Neodymium
Europium	Erbium	Gadolinium	Mercury	Iridium	Niobium
Lutetium	Molybdenum	Osmium	Palladium	Lead	Praseodymiu
Rhenium	Platinum	Rhodium	Rubidium	Ruthenium	Selenium
Thalium	Tungstn	Xenon	Terbium	Tantalum	Samarium
Ytterbium	Chromium				

(Protocol et al., 2010)

1.3.3 Sources of Heavy Metals:

There are different sources of heavy metals in the environment. These sources are large in numbers. These heavy metals can be natural or anthropogenic.

1.3.3.1 Heavy Metals from rocks and soil

One of the most principal and natural source of heavy metals are soil and rocks. Examples of rocks are: magmatic rocks, sedimentary rocks, metamorphic rocks (Bradl, 2005). Organic materials and the clay materials of the soil are responsible as the sources of heavy metals. Moreover the Oxides, oxyhydroxides and hydroxides of some metals, for example: Fe, Mn, and AI, can be an important element present in the soil.

1.3.3.2 Heavy Metals from water

Heavy metals can be generated from the surface water as well as from the ground water. The origins of the metals from groundwater are mentioned in Table 2 (Bradl, 2005).

Table 2 Sources of heavy metals

Sources	Inorganic Contaminant	Organic Contaminant
Agricultural areas	Heavy metals, salts	Pesticides
Urban areas	Heavy metals (Pd, Cd, Zn), salts	Oil products, Biodegradable organics
Industrial sites	Heavy metals, metalloids, salts	Polycyclic Aromatic Hydrocarbons (PAH), Chlorinated hydrocarbons
Landfills	Heavy metals, salts	Biodegradable products

Mining disposal sites	Heavy metals, salts	Xenobiotics
Dredged sediments	Heavy metals, salts	Xenobiotics
Hazardous waste sites	Heavy metals, salts	Concentrated Xenobiotics
Leaking storage tanks		Oil products
Line sources	Heavy metals (Cd, V, Pb), salts	PAHs, oils products, pesticides

(Bradl, 2005)

1.3.3.3 Heavy Metals from nature and anthropogenic sources

Today's environment has become contaminated with heavy metals due to smelting, industrial and mining activities in the more or less all the large and developing towns (Akoto, Bortey-sam, Ikenaka, Shouta, & Yohannes, 2017). Moreover, heavy metals can spread through different natural and anthropogenic sources. All the possible sources of these are mentioned in Table 3 (Singh et al., 2018).

Table 3 Natural and Anthropogenic sources of heavy metals

Natural Sources	Anthropogenic Sources
1. Weathering minerals	1. Pesticide, preservative, bio-solid ore mining and smelting (As)
2. Erosion ,Volcanic activity	2. Tannery, steel industry, mining, pesticide and fertilizer industry (Cr)
3. Forest fires and biogenic sources	3. Au-Ag mining, coal combustion and medical waste (Pb)

4. Vegetation	4. Paints, plastic stabilizers, electroplating, fertilizer.
5. Kitchen appliances, instruments used in surgery and batteries (Ni)	
6. Pesticide, fertilizer, bio-solid (Cu)	
7. Leaded fuel, battery wastes, insecticide and herbicide (Pb)	

(Singh et al., 2018)

1.3.4 Classification of Heavy metals

On the basis of toxicity heavy metals can be classified into four major groups. The highly toxic and less toxic heavy metals causes harm to our environment and to the living organisms. On the other hand there are some heavy metals present that are essential for the living of many organisms. Such as: Cu, Zn, CO, Cr, Mn and Fe. These metals are called micronutrient (Raskin & Ensley, 2000). The classification of heavy metals including their examples is given in table 4.

Table 4 Classification of heavy metals

Non essential Heavy Metals	Toxic heavy metal (Less)	Toxic Heavy Metals (High)
• Barium • Aluminum • Lithium • Zirconium	• Tin • Aluminium	• Mercury • Cadmium • Chromium

(Raikwar, Kumar, Singh, & Singh, 2008)

1.3.5 Toxicity of Heavy Metals

1.3.5.1 Toxicity to Human

Most of the heavy metals cause toxic effect to human.. They are widely spread throughout the environment. These metals are considered as most toxic elements for all the living organisms, including human and animals. These heavy metals are widely dispersed in the environment. (Morais, Garcia, & Pereira, 2012). These elements is no good for humans, and there is no homeostasis mechanism present for them (Jelescu & Dima, 2014). They can exert their toxic effects even at a low concentration. These metals are as diverse as their quantity in the environment (Morais et al., 2012). These toxic effects can be life threatening as well. The effects of various toxic heavy metals on human beings at a particular concentration are mentioned in table 5.

Table 5 Toxic effects of metals

Name	Limit(ppm)	Effects	Reference
As	0.01	Affects essential cellular processes. For example: oxidative phosphorylation and ATP synthesis.	(Appenroth, 2010)(Tripathi et al., 2007)
Cr	0.1	It causes Hair loss	(Farag, 2000)
Ag	0.1	Graying of skin, tissue and body, Breathing problems, Irritates throat and lung, Causes stomach	(Agency for Toxic Substances and Disease Registry (ATSDR), 1990)
Ni	0.2	Causes skin diseases such as allergy, itching, Lung cancer, Sinuses, immunotoxic,	(Duda-chodak &

		neurotoxic, genotoxic, affects fertility, Causes hair loss	Duda, 2014)
Zn	0.5	Dizziness, fatigue etc.	(Salzman, Smith, & Koo, 2002)
Hg	2.0	Autoimmune diseases, Depression, Drowsiness, Fatigue, hair loss, Insomnia, loss of memory, restlessness, disturbance of vision, tremors, temper outbursts, brain, damage, lung and kidney failure	(Gulati, Banerjee, Lall, & Ray, 2010)
Se	50	Affects the endocrine function, Impairs the activity of natural killer cells, Hepatotoxicity, gastrointestinal disturbances, Dizziness	(Vinceti, Wei, Malagoli, Bergomi, & Vivoli, 2001)
Pb	15	Impaired development of children due to, excessive explosure, reduced intelligence, short-term memory loss, disabilities in learning and coordination problems, a risk of cardiovascular disease	(Padmavathiamma & Li, 2014)
Cu	1.3	Brain and kidney damage, Excessive exposure causes liver cirrhosis and chronic anemia, Irritates stomach, intestine irritation	(Wuana & Okieimen, 2011)
Cd	5.0	Carcinogenic, Mutagenic, endocrine disruptor, lung damage, fragile bones, affects calcium	(Degraeve, 1981)

		regulation in biological systems	
Ba	2.0	Cause cardiac arrhythmias, respiratory failure, gastrointestinal dysfunction, muscle twitching, elevated blood pressure	(Jacobs, Taddeo, Kelly, & Valenziano, 2002)

(Singh et al., 2018)

Mainly at higher concentration most of the heavy metals exerts their toxic effects. On the other hand there are some heavy metals which can be harmful even at a lower temperature. The limit to which it is safe to intake heavy metals are mentioned in table 6 (Pandey Govind, 2014).

Table 6: The limit of safe intake of Heavy Metals

Heavy Metal	Daily Dose (µg/day)	
	Parenteral	Oral/Topical/Dermal/mucosal
Arsenic	1.5	15
Cadmium	0.5	5
Lead	1.0	10
Mercury	1.5	15
Chromium	25	250
Copper	250	2500
Manganese	250	2500
Molybdenum	25	250
Nickel	25	250
Palladium	10	100
Platinum	10	100

Vanadium	25	250
Osmium	10 (combination not to exceed)	10 (combination not to exceed)
Rhodium		
Ruthenium		
Iridium		

(Pandey Govind, 2014)

1.3.5.2 Toxicity to animals

As the population of this world is increasing, the pollutants such as radio nuclides, heavy metals, toxic inorganic and organic substances are increasing as well. At present, this has become a serious threat to all the living organisms of this world including the land animals and the aquatic animals (Pandey Govind, 2014). Heavy metals are the main pollutants to the aquatic organisms. The heavy metals get mixed with the aquatic system following various processes, including effluent, smelting, leaching and sewage of garbage. This produces severe harm to the aquatic organisms (Pandey Govind, 2014). The waste water that is released from the tannery affects the aquatic systems severely by poisoning the water. This is because the waste that is released from the tannery contains a large amount of chemicals that are harmful to the aquatic system. The unrestricted discharge of tannery wastes resulted in drastic health hazards to different organisms (Praveena, Sandeep, Kavitha, & K, 2013). Death of the aquatic animals might occur because of the nutrient pollutions. These include nitrogen, phosphates, etc. The chemical contamination results in declining the tadpole mass, frog biodiversity and oil pollution in the aquatic system. It results in the occurrence of the reproduction of the aquatic organisms. This also results in enhancing the susceptibility of these organisms to various dangerous diseases. It can cause liver and kidney damage, irritate

the gastro-intestinal tract, and can damage the nervous system as well (Pandey Govind, 2014).

One of the toxic heavy metals, for example Hg, can be toxic to animals and human being. this happens when they eat fishes that are grown in the water containing Hg. According to the 'Environmental Protection Agency' (EPA) of USA, strict precautions must be followed while disposing and handling those heavy metals (Pandey Govind, 2014). These metals are sometimes mixed with the fertilizers that are used in the agricultural purpose, which causes in severe health hazards to the animals and human being as a result of consuming them (Pandey Govind, 2014). The Sn is comparatively less toxic than other heavy metals. The existence of heavy metals in fish mainly depends on various physiological factors, such as the age of the fish. These fishes are the largest main sources of Hg and As for the human beings (Pandey Govind, 2014). The release of the heavy metals from industrial, domestic and man-made activities contaminates the aquatic organisms. These contaminations can destroy ecological balance. This may result in death of the aquatic species. The presence of As can be found in the air, soil, water and in all living tissues. It is considered as a carcinogen. It causes malformations and fetal death and malformations in most of the mammal species (Pandey Govind, 2014). As a result of increased use of metals, the amount of Cr in the environment is also increasing day by day. The Cr (VI) is the most toxic form of Chromium for humans and animals. This is a big threat to the growth development of the aquatic animals (Govind Pandey, 2004). The Hg has the capability of absorbing in the sea fish and water, which causes serious affects to the fish as well as to the food chain (Pandey Govind, 2014).

Animals are daily getting contaminated by heavy metals. For example Hg and Al two poisonous heavy metals are poisoning the animals through vaccines, polluted air and water. Used of leaded fuel are also causing health hazards of the animals. The toxic effects of these metals are: mutagenicity, tetratogenecity, carcinogenicity, immune-suppression and impaired

reproduction. Domestic and wild animals including the pets are also exposed to these dangerous environmental pollutants (Pandey Govind, 2014). A result of an experiment revealed that dairy cattle, laying chicken and growing swine poses the residues of Cd and Pb, which were received by them through the food products they were given. Both of these metals are able to get deposited in liver and kidney. On the other hand but Pb has the ability to get deposited in the bone as well. A moderate intake of Pd causes little or no effects to the animals; however the intake to Cd should must be avoided (Pandey Govind, 2014). High concentration of some heavy metals (Pb, Se and Hg) was found to be present in some of the wild species from the north-east of India. The elemental levels showing their toxicity or deficiency were found significantly. A study shows some behavioral abnormalities in those animals, for example, loss of appetite, salivation, tendency to move within a circle, constipation, photophobia, etc. (Pandey Govind, 2014).

1.3.6 Heavy Metal Resistance of Bacteria

There are many heavy metals that are necessary for the growth of the micro-organisms at a low concentration. Although at a higher concentration they can be fatal to these micro-organisms (Trevors, Oddie, & Belliveau, 1985). Bacteria that are evolved by tolerating high levels of heavy metals, results in developing a protection mechanism that helps them to get adapted with the high metal concentration environment. The metal tolerance of a certain bacteria depend on several factors, for example; it depends on the way by which metal transports in to the cell, the position of the metal resistance gene and also on the contribution of the metal ion in cellular metabolism. (Ianieva, 2018)

1.3.7 Heavy Metal resistance Mechanism

There are in total five ways of mechanism by which bacteria can grow resistance against heavy metals. A single bacterium can possess more than one protection mechanism. All the mechanisms are mentioned and described below in the following:

1. Extracellular barrier

2. Active transport of metal ions (efflux)

3. Extracellular sequestration

4. Intracellular sequestration

5.Reduction of metal ions (Harrison, Ceri, & Turner, 2007), (Choudhury & Srivastava, 2001)

1.3.7.1 Extracellular barrier

The extracellular layer works as a barrier for the bacteria to prevent the entry of the heavy metals. These extracellular barriers includes: the capsule, the plasma membrane or the cell the bacterium (Ianieva, 2018). The adsorption process of the bacteria is a passive process. Even the dead bacteria are also capable of adsorbing metal ions. Moreover, bacteria, those are killer by producing excessive temperature also showed the same or sometime better ability to bind with the metal ions (Ianieva, 2018). According to many living cells accumulates metal ions by following two steps. At first a initial and rapid non- specific adsorption is done by the cell wall and after that the metal ion transfers in to the cytoplasm by slow active transportation process. Through bacterial capsule, heavy metal ions can be absorbed. Extracellular biopolymers of some bacteria have shown the ability to accumulate metal ions, for example: *Enterobacter chloaceae*, *Klebsiella aerogenes*, *Marinobacter sp*, *Acinetobacter sp* (Ianieva, 2018). The biofilm of *Pseudomonas aeruginosa* showed has comparatively higher resistance to zinc, copper and lead than planktonic cells. On the other hands the cell

those were located at the periphery of the biofilm got killed. A study shows that, bacterial strains that are Copper-tolerant produces double EPS compared to the sensitive strains (Ianieva, 2018). The copper accumulation and the EPS production were induced by the copper ions. It was observed that the metal ions produced inhibitory effects in the synthesis of bacterial EPS. Furthermore, several mutants of *Sphingomona spaucimobilis* which are tolerant to copper were obtained in a study. EPS is a process which is highly energy consuming (Ianieva, 2018). The increase of the copper tolerance of the mutants might be as a result of the declined growth rate of the bacteria and the saving of the energy for using them in the purpose of establishing protection against the metal stress (Ianieva, 2018). If the permeability of the plasma membrane of the bacteria changes, the bacterial ability to prevent the metal ions can also be changed. It can result in preventing the entry of the metal ions into the bacterial cell. E-coli mutants are lack of membrane proteins – porins that plays a role as channels. These membrane proteins help in the accumulation of low level of silver ions in the cell (Ianieva, 2018).

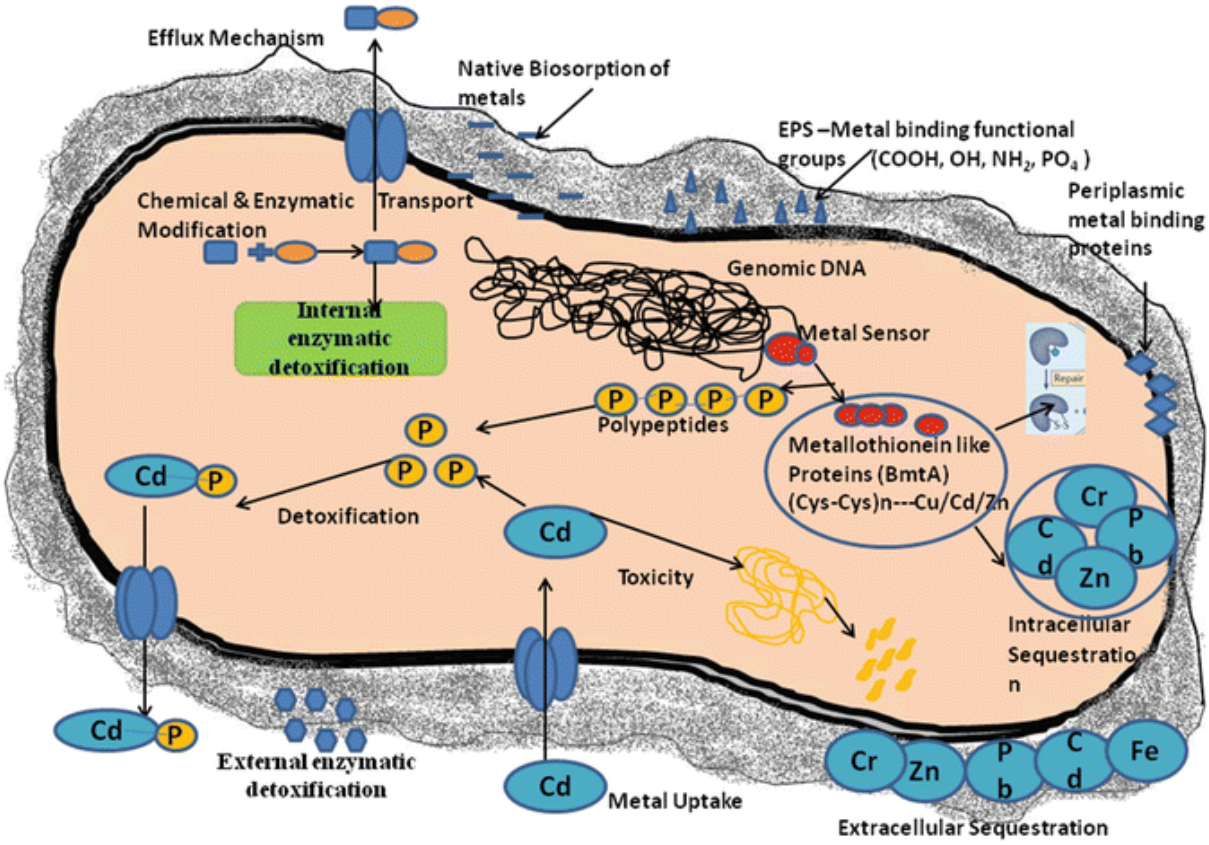

Figure 1: Heavy metal resistance of bacteria by forming extracellular barrier. (Bramhachari & Nagaraju, 2017)

1.3.7.2 Active transport of metal ions (efflux)

A large number of bacteria achieve resistance against heavy metals by following this mechanism (Ianieva, 2018). The genes that are responsible for efflux system are located in the chromosome and in the plasmid. The metals can enter the bacterial cell through some systems that are responsible for the transportation of some important elements such as; the chromate ions can easily enter the bacterial cell through suphate transporter system (Ianieva, 2018). On the other hand ions of zinc, cadmium, cobalt, manganese and nickel can enter the bacterial cells of *Ralstonia metallidurans* with the help of using magnesium transport system. Proteins are involved in the efflux system. They belong to CDF (cation diffusion facilitator),RND (resistance, cell division, nodulation) and P-type ATPases families (Ianieva,

2018). From these three protein families, both the CDF proteins and P-type ATPases are able to transport specific elements in the periplasm via the plasma membrane (Ianieva, 2018). The ions that have high affinity to the sulphar groups can transfer predominantly through P- type ATPases (Ianieva, 2018). These ions are Cu+, Ag+, Zn2+, Cd2+, and Pb2+. On the other hand, the divalent metal ions interact with CDF-proteins, for example, Zn2+, Ni2+, Co2+, Fe2+ and Cd2+. The transport complexes that are formed by the RND- proteins help to transport cations from the periplasm to the plasma membrane (Ianieva, 2018).

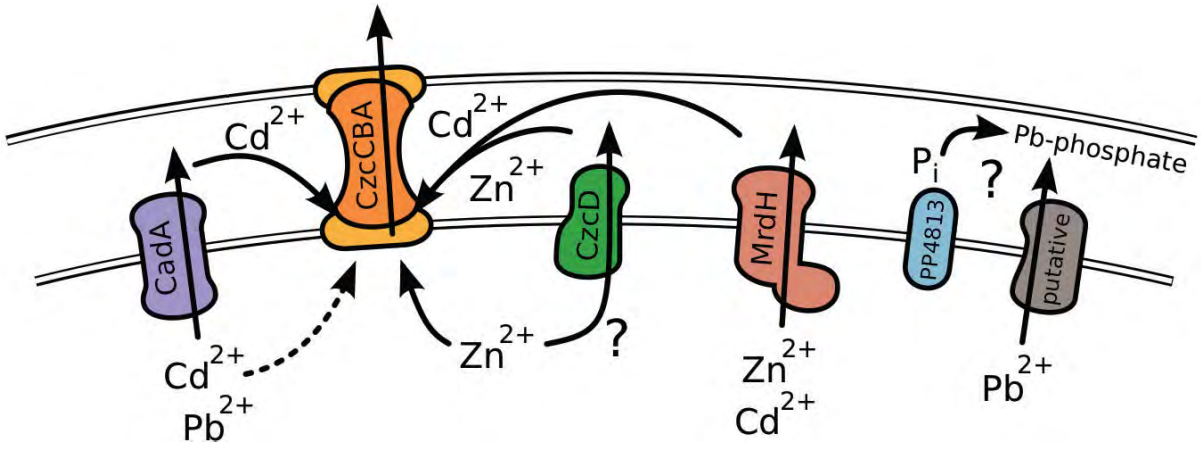

Figure 2: Zn2+,Pb2+ and Cd2+ resistance in Pseudomonas putida KT2440. Here, Cd2+ has been removed from the given cell by CadA and CzcCBA. On the other hand, Zn2+ is exported efficiently by CzcCBA (Hynninen, 2010)

1.3.7.3 Intracellular sequestration

Intracellular sequestration is a process where the metal ions form complexwith different compounds in the cytoplasm of the cell (Ianieva, 2018). Phytochelatins and metallothioneins are the two classes of peptides that are eukaryotic and bind with metal ions. These peptides contains high amount of cystein residues (Ianieva, 2018). They help to bind the metal ions with the sulfhydryl. Phytochelatins are found in plants and fungi; their molecular-weight is comparatively lower. PCC7942 is a type of peptide that contains fewer amounts of cysteine residues compared to the analogous eukaryotic peptide (Ianieva, 2018).

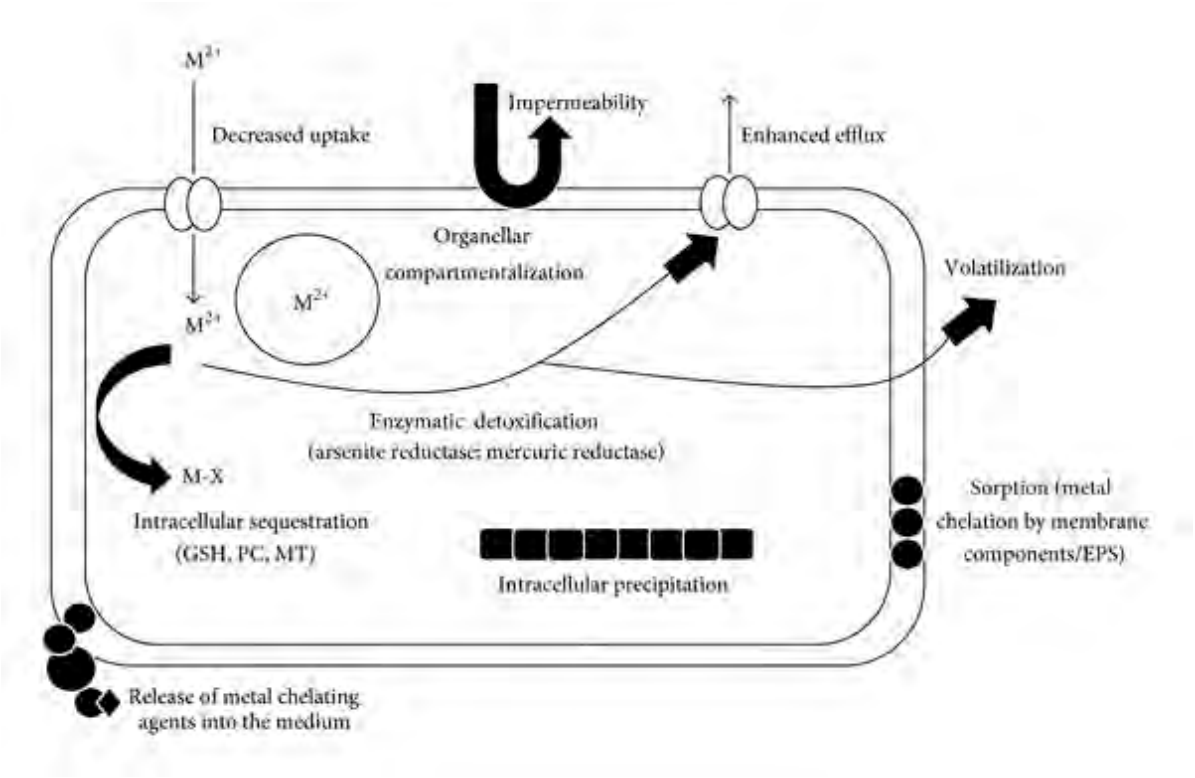

Figure 3: An intracellular sequestration mechanism is adapted by bacteria for the purpose of metal resistance (Srivastava & Kowshik, 2013)

1.3.7.4 Extracellular sequestration

Extracellular sequestration is a process where metal ions accumulate by cellular components at the site of periplasm or in the outer membrane or it builds complex as insoluble compounds (Ianieva, 2018). *Pseudomonas syringae* that are copper resistant strains synthesizes proteins that are copper inducible. These proteins are CopA, CopB (periplasmic proteins) and CopC (outer membrane protein) (Ianieva, 2018). They help to bind bacterial colonies and copper ions and turn it to blue after accumulation. The same kind of blue colonies of bacteria could be seen at the time of growth of *Pseudomonas pickettii* US321 (Ianieva, 2018). This bacterium is copper-tolerant. This is because the bacterial were accumulated with the copper ions in the outer membrane or in the periplam. According to Authors the resistant strain formed complex by accumulating with copper and then transported it within the cytoplasm

(Ianieva, 2018). On the other hand sensitive strain got accumulated copper as a ionic form. This ionic form is free and highly toxic for the cell (Ianieva, 2018).

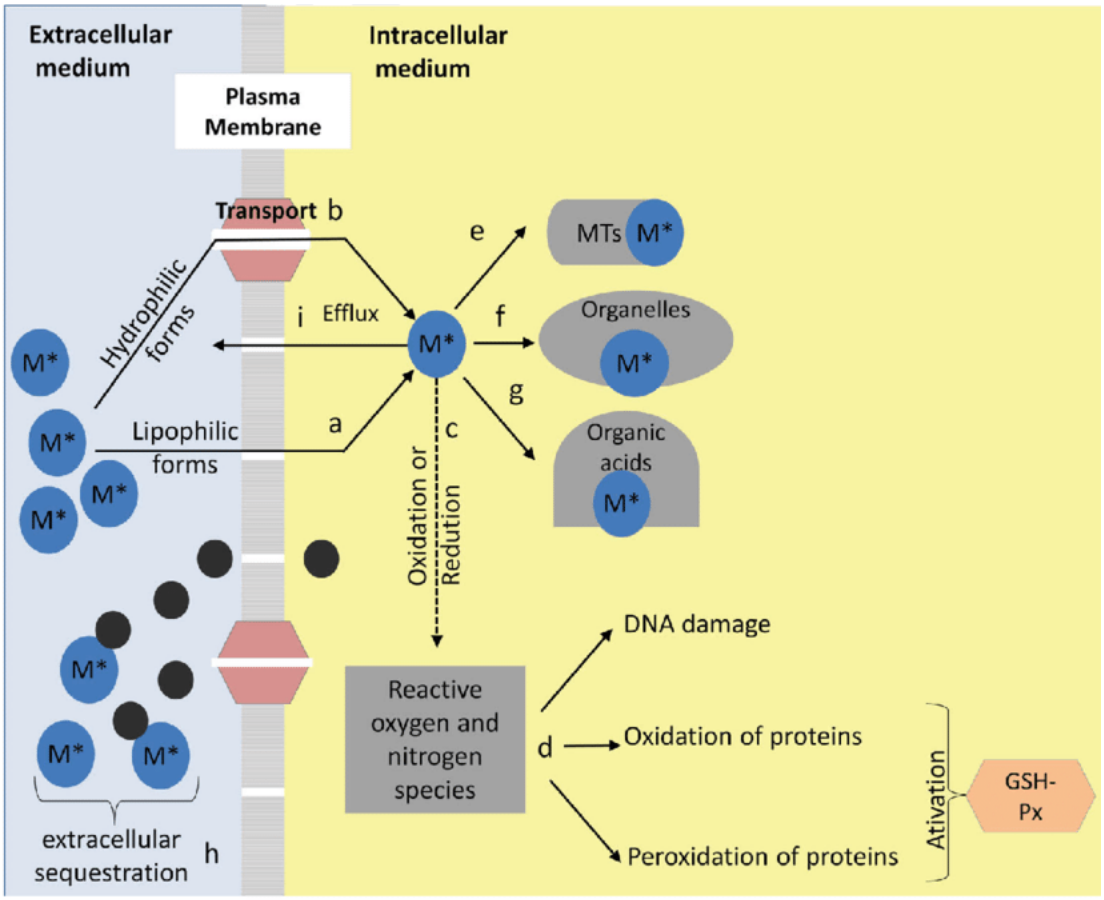

Figure 4: Heavy metal resistance by extracellular sequestration mechanism. Bacteria create complex with extracellular medium (h) by which metals are expelled out of the cells (de Paiva Magalhães, da Costa Marques, Baptista, & Buss, 2015).

1.3.7.5 Reduction of heavy metal ions by bacteria

A large number of heavy metals can be reduced by bacteria. The name of those bacteria and their reduction process is shown in table (Ianieva, 2018). In purpose of generating energy some bacteria uses metalloids and metals as the acceptor or donor of electrons. During bacterial anaerobic respiration, the oxidized form of metal can serve as the terminal electron acceptors (Ianieva, 2018). In the formation of comparatively less toxic form to chromium and mercury, enzymatic reduction of metal ions occurs (Ianieva, 2018).

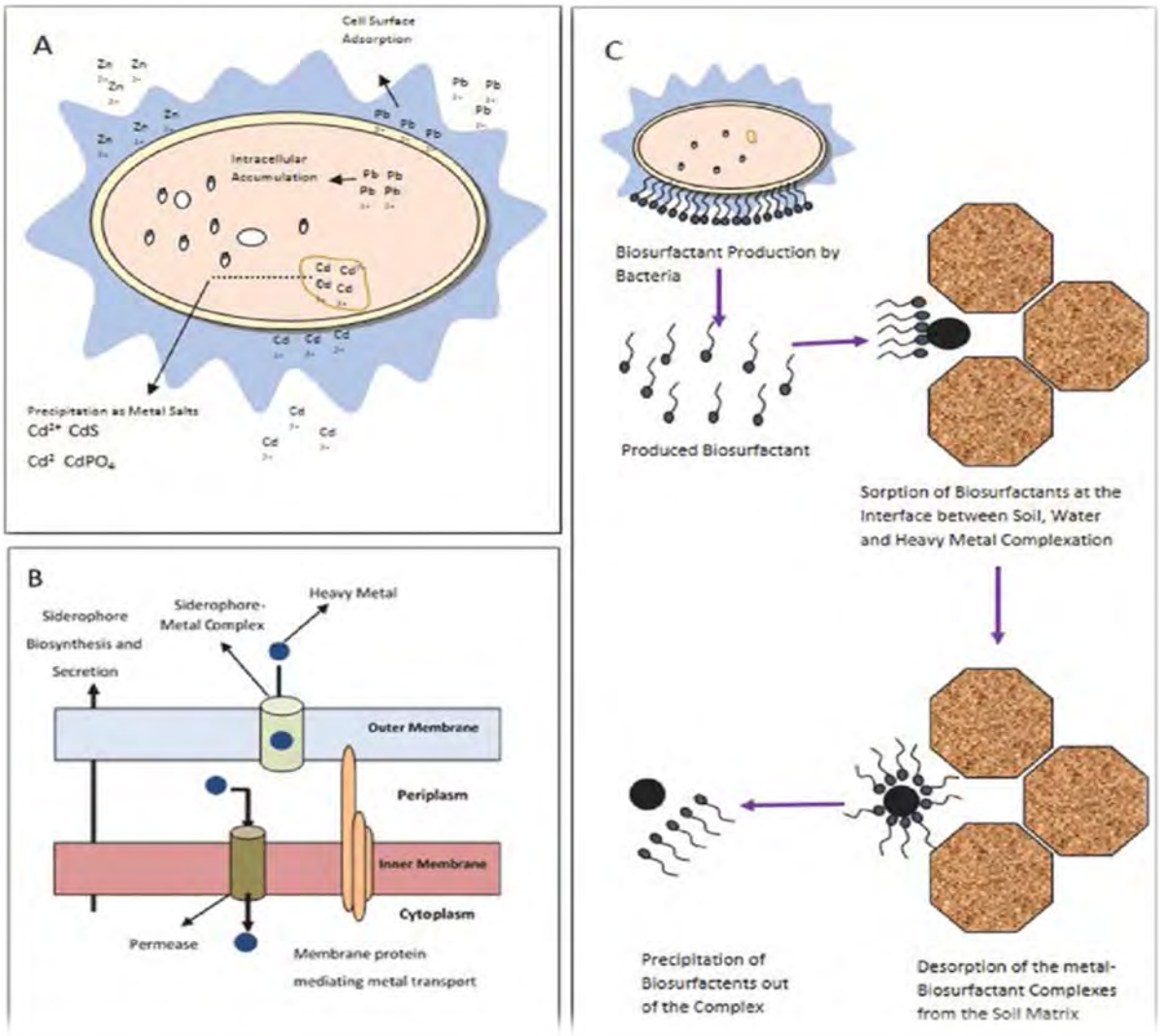

Figure 5: Bacterial bioremediation. (A) Biosorption of the heavy metals by bacteria through cell surface adsorption, intracellular accumulation and extracellular precipitation. (B) Bacterial heavy metal reduction through the siderophore formation. (C) Heavy metal reduction of bacteria through bio-surfactant production (Banik, Das, Islam, & Salimullah, 2014)

The reduction processes and the name of the micro-organisms used in it are given in table 7.

Table 7: Micro-organisms used in reduction process

Reduction process	Microorganism
Hg2+/Hg0	*Bacillus cereus* *Klebsiella pneumoniae* *Pseudomonas stutzeri*
Fe3+/Fe2+	*Geobacter metallireducens* *Bacillus thermoamylovorans*

Cr6+/Cr3+	*Desulfomicrobium norvegicum* *Ochrobacterium intermedium*
As5+/As3+	*Staphylococcus aureus*
U6+/U4+	*Desulfovibrio desulfuricans* *Shewanella putrefaciens* *Thermoterrabacterium ferrireducens*
Mn4+/Mn2+	*Shewanella putrefaciens*
Se6+/Se4+/Se0 Se4+/Se0	*Ralstonia metallidurans* *acillus. thermoamylovorans* *Shewanella oneidensis*
V5+/V4+	*Shewanella oneidensis* *Geobacter metallireducens*
Tc7+/Tc4+	*Geobacter sulfurreducens* *Shewanella putrefaciens*
Mo6+/Mo5+	*Thiobacillus ferrooxidans*
Au3+/Au0	*Strenotrophomonas sp.*
Te4+/Te0	*Bacillus thermoamylovorans* *Sewanella oneidensis*

1.3.8 Introduction of Cadmium

Cadmium is one of the heavy metals which have a high toxicity. It is toxic at a very low concentration and also has acute as well as chronic effects on the human health and on the environment (A/S, 2003). Cadmium is non-degradable in the nature. So when cadmium is release once in the environment then it remains unchanged and dispersed in the air. Cadmium is relatively sluble than other heavy metals (A/S, 2003). For this reason they tend to move more. They are comparatively more bio-avalable and they have a tendency to bio-accumulate as well.

1.3.9 Historical perspective of Cadmium

Cadmium is malleable, ductile, soft and bluish white metal. Cadmium was discovered first in Germany in 1817 (Thomas o. Llewellyn, 1994). In the later 19th century Germany produce their 1st commercial cadmium metal. Until First World War, Germany was the only important producer of this metal. Germany used to as a by-product during the smelting of Cadmium bearing zinc ores of Upper Silesia. In 1907, United States started producing Cadmium. It began when a company named Grasseli Chemical of Cleveland, as a by-product of zinc smelting had recovered the metallic cadmium (Thomas o. Llewellyn, 1994). About 85% of the cadmium from the zinc concentrates was lost before this, as a result of the fractional distillation of the zinc metal. From 1990's Germany started to export cadmium as cadmium sulfide pigment and as metallic stick (Thomas o. Llewellyn, 1994). After First World War, the production of cadmium from Germany was curtailed by 1917. After 1917 United States was in number one position in producing cadmium metal and held that position for almost 50 years as well. From 1979 to 1989 the total consumption of cadmium in United State was 3880 ton each year. As it was never recycled until today, it is thought to be dispersed in our surrounding environment (Thomas o. Llewellyn, 1994).

Cadmium is comparatively a rare element. In the earth's crust its concentration is lesser than both the Hg and Pb (Effects & Mercury, 1972). Cadmium is present in the seawater in a very small amount. It is present in a very trace amount in a large range of plant and animals (Effects & Mercury, 1972)

1.3.10 Uses of Cadmium:

The main use of cadmium is described below in the table.

Table 8: Uses of Cadmium

Chemical form		Main uses
Cadmium metal		Battery cell (electrode), electric appliance, gilt
Cadmium compounds	Cadmium sulfide	Yellow pigment (cadmium yellow)
	Cadmium stearate	Stabilizer (polyvinylchloride)
	Cadmium acetate	Glaze of ceramic ware
	Selenium cadmium	Semiconductor, pigment (cadmium red)
	Cadmium chloride	Photography dye stuff

(Nakamura, Kinoshita, & Takatsuki, 1996)

1.3.11 Toxicity of Cadmium:

Cadmium is one of the heavy metals, which can exert its toxic effect in higher concentration, sometimes in low concentration. Cadmium becomes toxic and causes danger to the human health because of long term exposure in the human body. It can affect the kidney and bone, lung, ocular tissue, periodontal tissue, mammary gland, can accelerate the occurrence of diabetes and hypertension even can cause cancer as well.

1.3.11.1 Kidney and bone:

Long time exposure to high concentration of cadmium may result in Itai-Itai disease (Inaba et al., 2005). According to a study of 1960s, this disease can occur if someone takes cadmium more than 600µg/day (Kobayashi et al., 2006) (Satarug, Garrett, Sens, & Sens, 2010). This disease mainly occurs in women and it affects the tubular and glomerular function (Satarug et al., 2010). Long term exposure of low dose of cadmium cause tubular malfunction as well as it destroys the absorption capacity of vitamins, nutrients and minerals. It might also cause abnormal urinary excretion and may result in kidney damage (Satarug et al., 2010).

1.3.11.2 Diabetes

Cadmium is also responsible for increased rate of tubular impairment in the diabetes patient (Schwartz, Il'yasova, & Ivanova, 2003). A study has been done on a Chinese patient who already had type 2 diabetes and it is found in the result that the risk of tubular impairment has been increased (Satarug et al., 2010). Low level of cadmium thus plays a vital role of risk factor in patient suffering from diabetes or pre-diabetes (Satarug et al., 2010).

1.3.11.3 Hypertension

A dose response relationship has been found between hypertension and urinary cadmium. In Korea, 26.2 % of their people have hypertension (Eum, Lee, & Paek, 2008) (Satarug et al., 2010). Among these people most of them were found to be having comparatively higher level of cadmium in the urinary. Moreover, this cadmium blood pressure was strongly be seen in the nonsmokers, immediately be seen on the former smokers and very few or was absent among the smokers (Satarug, Nishijo, Ujjin, Vanavanitkun, & Moore, 2005) (Satarug et al., 2010).

1.3.11.4 Blood vessels and the heart

A study has shown a link between cadmium levels in human body with high risk of PAD (Satarug et al., 2010) and MI. PAD risk was found to be 4.13 fold higher in the smokers compared to the person who doesn't smoke (Navas-Acien et al., 2009). This proves the key contribution of cadmium in elevating the risk of PAD. Moreover, high risk of myocardial infarction was observed in women when the urinary cadmium > 0.88 µg/g creatinine was compared with < 0.43 µg/g creatinine (Everett & Frithsen, 2008) (Satarug et al., 2010). It proves the toxic effects of cadmium on the blood vessels and on the heart as the smokers were inhaling a a particular amount of cadmium through smoke.

1.3.11.5 Lung

A sample group of 96 people (men) went through a study where 2 or 3 lung tests were done (Lampe et al., 2008), (Satarug et al., 2010). The result of the test showed reduced lung function in the men who were smokers compared to the men who were non-smokers. This study actually proves that lung diseases that are normally seen in the people who smoke might be because of the high cadmium level as urinary cadmium level increases when a person smokes (Satarug et al., 2010).

1.3.11.6 Periodontal Tissues

A high amount of urinary cadmium (3-fold) increase has been found to be associated with a 54% higher ratio for periodontal disease. For example, in a study it is found that among a group of adults, 15.4% had been suffering with periodontal disease (Arora, Weuve, Schwartz, & Wright, 2009) (Satarug et al., 2010). The mean urinary cadmium for the adults having periodontal disease was 0.50 µg/g creatinine. On the other hand, 0.30 µg/g creatinine was found for unaffected individuals (Satarug et al., 2010).

1.3.11.7 Ocular Tissues

High level of urinary cadmium was found to be present, associated with AMD among smokers (Erie, Good, Butz, Hodge, & Pulido, 2007). According to a study, urinary cadmium level (median) in former and current smokers with AMD was 1.18 µg/g creatinine (Satarug et al., 2010). Compared to the smokers without AMD, nonsmokers with AMD, and nonsmokers without disease, it was a lot higher, that is: 1.97-fold, 2.03-fold, and 2.07-fold higher respectively. Moreover, high retinal cadmium content was also observed in a study that was done on male, who were associated with AMD as well. (Satarug et al., 2010) (Wills et al., 2009) (Wills et al., 2008).

1.3.11.8 Mammary Gland

A study showed that samples of breast milk of some Austrian subjects contained cadmium content of 0.086µg/L on average (Gundacker et al., 2007) (Satarug et al., 2010).This level was comparatively lower in the nonsmokers who took mineral and vitamin supplements (Satarug et al., 2010). On the other hand, in Bangladesh, when the breast milk was checked, it is found that in breast milk from Bangladeshi subjects the average cadmium level was 1.6-fold higher than the Austrian subjects (Kippler et al., 2009). The findings show a key interference of cadmium in transportation and secretion of mammary gland (Satarug et al., 2010).

1.3.11.9 Cancer

There are several studies that prove the role of cadmium in the occurrence of cancer to the people who live in a place where there is high amount of cadmium concentration. Cadmium has various evidence of causing lung cancer. In the Kakehashi cohort, among women 2.5-fold high cancer mortality was found associated with tubular impairment (Nakagawa et al., 2006). Increased mortality from nephritis, nephritis, heart failure, and brain infarction was also observed in women and men (Satarug et al., 2010). Another study showed increased mortality risks by 25% and 33% among the people having 2-fold increased cadmium concentration in blood who live in low and high-exposure areas, respectively (Nawrot et al., 2008).

Chapter 2

MATERIALS AND METHODS

2.1 Introduction

In this part, the chemicals, reagents and equipment which are used in this study will be discussed. Besides that, in this section, the procedure of the full experiment will be discussed in details. Such as collection of the sample, different test which was done by the isolated sample, identification of the sample these all will be discussed.

2.2 Chemicals

All the chemicals or reagents that were used in this study are given below:

1. Nutrient Agar (NA)
2. Nutrient Broth
3. NaCl
4. Cadmium Sulphate Hydrate

2.3 Glassware and instruments

Instruments and glassware which was used throughout the experiment are listed in table

Table 9: Name and function of the machines that are used

Name of instrument	**Function**
Autoclave machine	Sterilization
Incubator	Incubation of solid culture mediums
Electric balance	Weight measurement

Laminar Air Flow	To maintain the aseptic environment
Digital Shaking incubator	Incubation of liquid culture mediums
UV-Vis Spectrophotometer	Measurement of absorbance
SIEMENS Up-ride Freezer	For storing bacterial culture stock
Vortex Mixer	For proper mixing
Electronic Centrifuge	Collection of supernatant
Micropipette	For withdrawing reagent and media in trace amount

2.4 Collection of sample

There are 230 small and large rivers in Bangladesh. Buriganga is one of those rivers (Uddin, 2018). It is situated beside on the south side of Dhaka city and is the polluted most river in our country. The reason behind this is most of the industrial wastage and domestic wastes are dumped in it every year. The river is now biologically and hydrogically dead. 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). There are around 343 tanneries established beside the bank of Buriganga and from these industries approximately 21,600 square meters of waste is released every day. Around 343 tanneries is situated on the bank of the river Buriganga and from these tanneries every day 21,600 square meters of wastewater is released to the river water. These waste contains huge amount of heavy metals for example, chromium, lead, ammonium, sulphur, cadmium, salts etc which get mixed with the water of Buriganga and make it more polluted (Uddin, 2018). 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 our samples from three different regions of

Buriganga River. The three different locations are: Showarighat, Pargandaria and Faridabadh. We have collected the soil sample from Showarighat and Pargandaria.

2.5 Isolated Bacterial strains:

An isolation process was previously done on the sample to isolate the chromium resistant bacteria. 14 different types of chromium resistant bacteria were previously isolated. Our work was on these chromium resistant bacteria to check if they are cadmium resistant or not and the analyzation of the MIC of cadmium to these bacteria. Different name tags were given to the bacteria, such as; A1, A2, B1, B2, C1, C2, D1, D2, E1, E2, F1, F2, G1, G2.

2.6 Preparation of Broth solution:

Solution of nutrient broth was prepared to use at the time of sub-culturing the bacteria. This broth is further needed to test the MIC of the 14 isolated bacterial strains. To make the nutrient broth according to the instruction given in the packaging, 13g of nutrient broth powder was taken in 1000mi of distilled water.

2.7 Preparation of Saline Water (0.9% NaCl):

0.9% of NaCl solution was used at the time of dilution of the bacterial strains. The dilution was done to get an appropriate result and proper growth of bacteria in the MIC test. In this case 9g of NaCl salt was taken in a conical flask and with it distilled water was poured up to 1000ml to make the saline water.

2.8 Preparation of 1M salt solution

The molecular weight of the cadmium salt that was taken was 769.51. To prepare the cadmium salt solution 7.6951g of cadmium sulfate hydrate salt was taken to prepare 10ml salt solution each day of the MIC test.

2.9 Preparation of salt-broth solution of different concentration

This prepared cadmium salt solution was further used to prepare salt containing broth where the minimum inhibitory resistance of the bacterial strains was tested. Measurements of the ingredients in some of the different concentrations of salts are given table 9.The total solution containing broth and salt was of 15ml as each concentration was tested 3 times for each bacterial strain and 5ml of salt-broth solution was taken in each test-tube where the MIC was measured.

Table 10: Preparation of different concentration of salt solution

Concentration	Measurement of salt	Amount of Broth	Total 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
20mM	300µL	14.700ml	15ml

2.10 Minimum Inhibitory Concentration (MIC) determination

Minimum inhibitory concentration (MIC) means the lowest concentration of heavy metals that inhibit the growth of bacteria. (Baz.S et al, 2014). To determine the MIC, bacteria was incubated in the nutrient broth media at different concentration of cadmium sulfate (5mM to 30mM). Before incubation of bacteria, 10 times serial dilution was done of the bacteria in saline water. 50µL of the diluted bacterial culture in saline was added to the nutrient broth by

using a micropipette and 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.

2.10.1 Pre-MIC test process

DAY1

All the bacteria were cultured in agar plates. For this, 350ml agar media was prepared where 9.8 gm of Nutrient agar was mixed with distilled water in a conical flask and it was poured up to 350ml. 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. After the autoclave was done agar plates were prepared 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.

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.

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. 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.

2.10.2 Measurement of the MIC of the isolated bacteria

In different test-tubes 5ml of salt-broth solution was taken in different concentrations (5mM to 30mM). In each test-tube 50µL of bacterial culture was incubated. Before the incubation of the bacteria, the bacterial culture was diluted 10 times in saline water (0.9% NaCl). The 50µL of diluted bacterial culture was added to the prepared nutrient broth by using a micropipette. All the test tubes that contained the bacterial culture were kept into the shaking incubator at 37˚C for 24 hours. The following day, absorbance of the incubated bacteria was measured at 600nm to determine the growth. According to the absorbance, no bacterial growth in a particular concentration was the minimum inhibitory concentration for that particular bacterium.

Chapter 3

RESULTS AND DISCUSSION

3.1 Results

3.1.1 Minimum Inhibitory Concentration of Cadmium to inhibit the growth of Cadmium resistant bacteria A1

According to the procedure that is described in the section 2.10, the Minimum Inhibitory Concentration of the isolated sample A1 was determined and the obtained result is given below in the following table.

Table 11: MIC of Cadmium resistant organism A1 against different Cadmium concentration

Cd Concentration	Absorbanceat 600nm Test Tube 1	Absorbanceat 600nm Test Tube2	Absorbance at 600nm Test Tube3	Average	Standard Deviation
3mM	0.035	0.041	0.038	0.038	0.003
5mM	0.039	0.028	0.027	0.031333	0.006658328
7mM	0.022	0.019	0.029	0.023333	0.005131601
10mM	0.017	0.019	0.015	0.017	0.002
13mM	0.001	0.008	0.005	0.004667	0.003511885
14mM	0	0	0	0	0
15mM	0	0	0	0	0

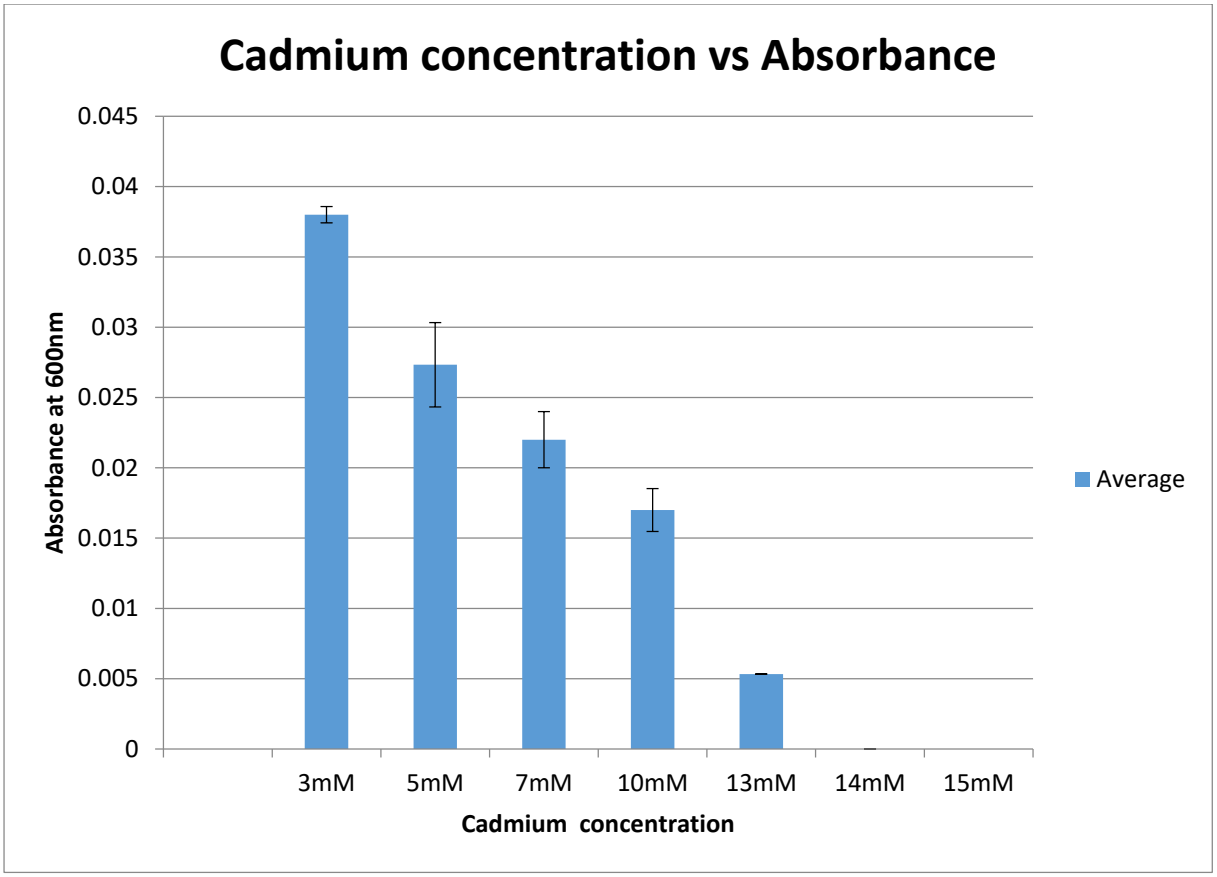

Figure 6: Minimum inhibitory concentration of the isolated sample A1

In the figure, it was observed that the isolated bacterium A1 has the ability to tolerate Cadmium concentration up to 13mM. So, it can be said that A1 was capable to demonstrate resistance till the Cadmium concentration of 13mM. However, from 14mM concentration, no growth of A1 was observed. Therefore, the Minimum Inhibitory Concentration for the A1 isolate is 14mM.

3.1.2 Minimum Inhibitory Concentration of Cadmium to inhibit the growth of Cadmium resistant bacteria A2

According to the procedure that is described in the section 2.10, the Minimum Inhibitory Concentration of the isolated sample A1 was determined and the obtained result is given below in the following table.

Table 12: MIC of Cadmium resistant organism A2 against different Cadmium concentration

Cd Concentration	Absorbance at 600nm Test Tube 1	Absorbance at 600nm Test Tube2	Absorbance at 600nm Test Tube3	Average	Standard Deviation
3mM	1.0125	0.994	0.998	1.0015	0.00973396
5mM	0.816	0.824	0.82	0.82	0.004
7mM	0.712	0.736	0.726	0.724667	0.01205543
10mM	0.704	0.702	0.708	0.704667	0.00305505
13mM	0.609	0.603	0.593	0.601667	0.0080829
15mM	0.35	0.359	0.357	0.355333	0.00472582
17mM	0.157	0.159	0.153	0.156333	0.00305505
19nM	0.009	0.01	0.007	0.008667	0.00152753
20mM	0	0	0	0	0
21mM	0	0	0	0	0

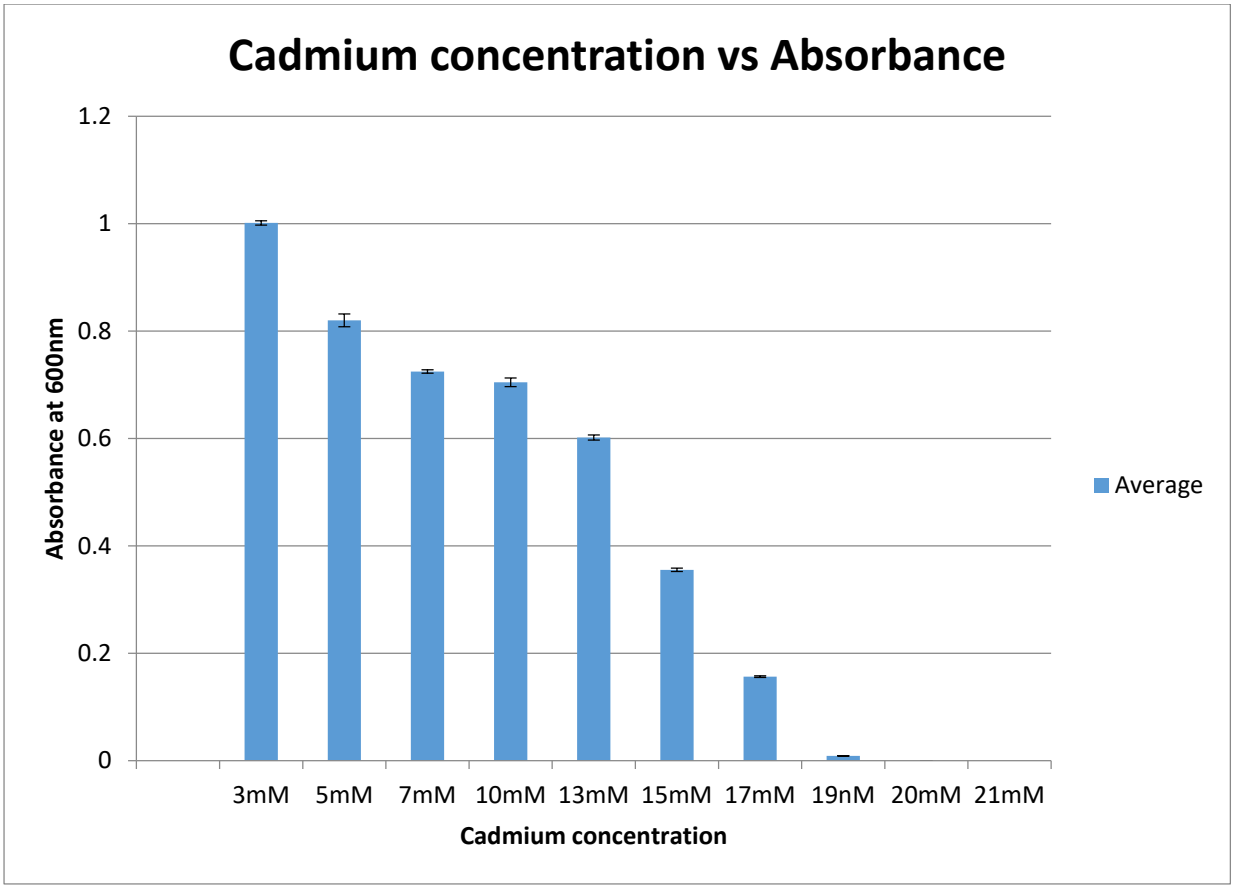

Figure 7: Minimum inhibitory concentration of the isolated sample A2

In the figure, it was observed that the isolated bacterium A2 has the ability to tolerate Cadmium concentration up to 20mM. So, it can be said that A2 was capable to demonstrate resistance till the Cadmium concentration of 19mM. However, from 20mM concentration, no growth of A2 was observed. Therefore, the Minimum Inhibitory Concentration for the A2 isolate is 20mM.

3.1.3 Minimum Inhibitory Concentration of Cadmium to inhibit the growth of Cadmium resistant bacteria B1

According to the procedure that is described in the section 2.10, the Minimum Inhibitory Concentration of the isolated sample B1 was determined and the obtained result is given below in the following table.

Table 13: MIC of Cadmium resistant organism B1 against different Cadmium concentration

Cd Concentration	Absorbance at 600nm Test Tube 1	Absorbance at 600nm Test Tube2	Absorbance at 600nm Test Tube3	Average	Standard Deviation
3mM	0.6	0.598	0.591	0.59633333	0.00472582
5mM	0.542	0.539	0.541	0.54066667	0.00152753
7mM	0.52	0.529	0.531	0.52666667	0.00585947
10mM	0.464	0.469	0.467	0.46666667	0.00251661
13mM	0.38	0.392	0.401	0.391	0.01053565
15mM	0.355	0.358	0.359	0.35733333	0.00208167
17mM	0.321	0.319	0.317	0.319	0.002
20nM	0.292	0.287	0.289	0.28933333	0.00251661
25mM	0.187	0.196	0.192	0.19166667	0.00450925
27mM	0.01	0.002	0.005	0.00566667	0.00404145
28mM	0	0	0	0	0
29mM	0	0	0	0	0

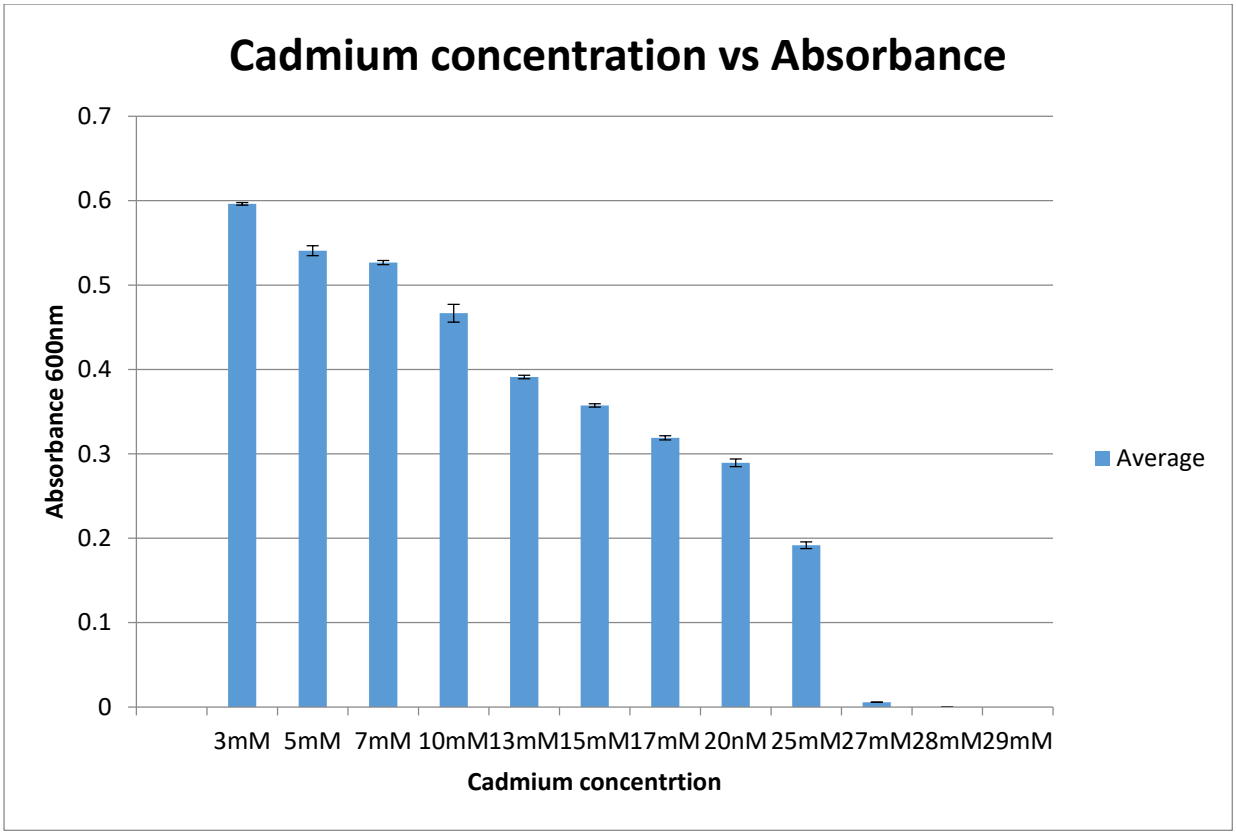

Figure 8: Minimum inhibitory concentration of the isolated sample B1

In the figure, it was observed that the isolated bacterium B1 has the ability to tolerate Cadmium concentration up to 27mM. So, it can be said that B1 was capable to demonstrate resistance till the Cadmium concentration of 27mM. However, from 28mM concentration, no growth of B1 was observed. Therefore, the Minimum Inhibitory Concentration for the B1 isolate is 28mM.

3.1.4 Minimum Inhibitory Concentration of Cadmium to inhibit the growth of Cadmium resistant bacteria B2

According to the procedure that is described in the section 2.10, the Minimum Inhibitory Concentration of the isolated sample B2 was determined and the obtained result is given below in the following table.

Table 14: MIC of Cadmium resistant organism B2 against different Cadmium concentration

Cd Concentration	Absorbance at 600nm Test Tube 1	Absorbance at 600nm Test Tube2	Absorbance at 600nm Test Tube3	Average	Standard Deviation
3mM	0.604	0.605	0.609	0.606	0.00264575
5mM	0.527	0.535	0.529	0.53033333	0.00416333
7mM	0.52	0.511	0.514	0.515	0.00458258
10mM	0.509	0.491	0.502	0.50066667	0.00907377
13mM	0.346	0.365	0.355	0.35533333	0.00950438
15mM	0.314	0.323	0.319	0.31866667	0.00450925
17mM	0.271	0.273	0.28	0.27466667	0.00472582
20nM	0.269	0.26	0.271	0.26666667	0.00585947
25mM	0.121	0.119	0.131	0.12366667	0.0064291
26mM	0.002	0.01	0.002	0.00466667	0.0046188
27mM	0	0	0	0	0
28mM	0	0	0	0	0

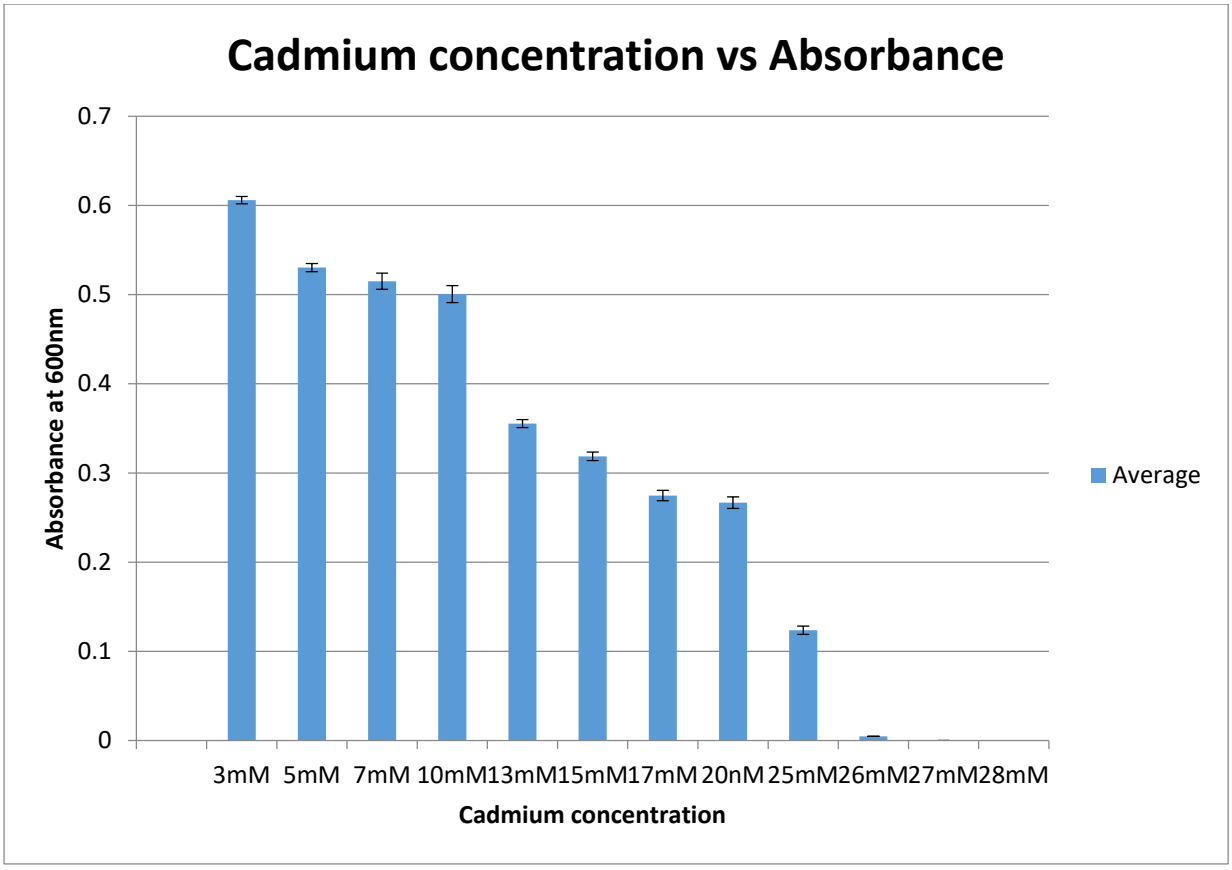

Figure 9: Minimum inhibitory concentration of the isolated sample B2

In the figure, it was observed that the isolated bacterium B2 has the ability to tolerate Cadmium concentration up to 26mM. So, it can be said that B1 was capable to demonstrate resistance till the Cadmium concentration of 26mM. However, from 27mM concentration, no growth of B1 was observed. Therefore, the Minimum Inhibitory Concentration for the B2 isolate is 27mM.

3.1.5 Minimum Inhibitory Concentration of Cadmium to inhibit the growth of Cadmium resistant bacteria C1

According to the procedure that is described in the section 2.10, the Minimum Inhibitory Concentration of the isolated sample B2 was determined and the obtained result is given below in the following table.

Table 15: MIC of Cadmium resistant organism B2 against different Cadmium concentration

Cd Concentration	Absorbance at 600nm Test Tube 1	Absorbance at 600nm Test Tube2	Absorbance at 600nm Test Tube3	Average	Standard Deviation
3mM	0.426	0.453	0.437	0.43866667	0.01357694
10mM	0.37	0.382	0.379	0.377	0.006245
15mM	0.324	0.312	0.309	0.315	0.00793725
17mM	0.259	0.261	0.256	0.25866667	0.00251661
20mM	0.199	0.209	0.203	0.204	0.00793725
25mM	0.143	0.139	0.14	0.14066667	0.00208167
30mM	0.092	0.097	0.095	0.09133333	0.00404145
34nM	0.006	0.008	0.01	0.038	0.05370289
35mM	0	0	0	0	0
36mM	0	0	0	0	0

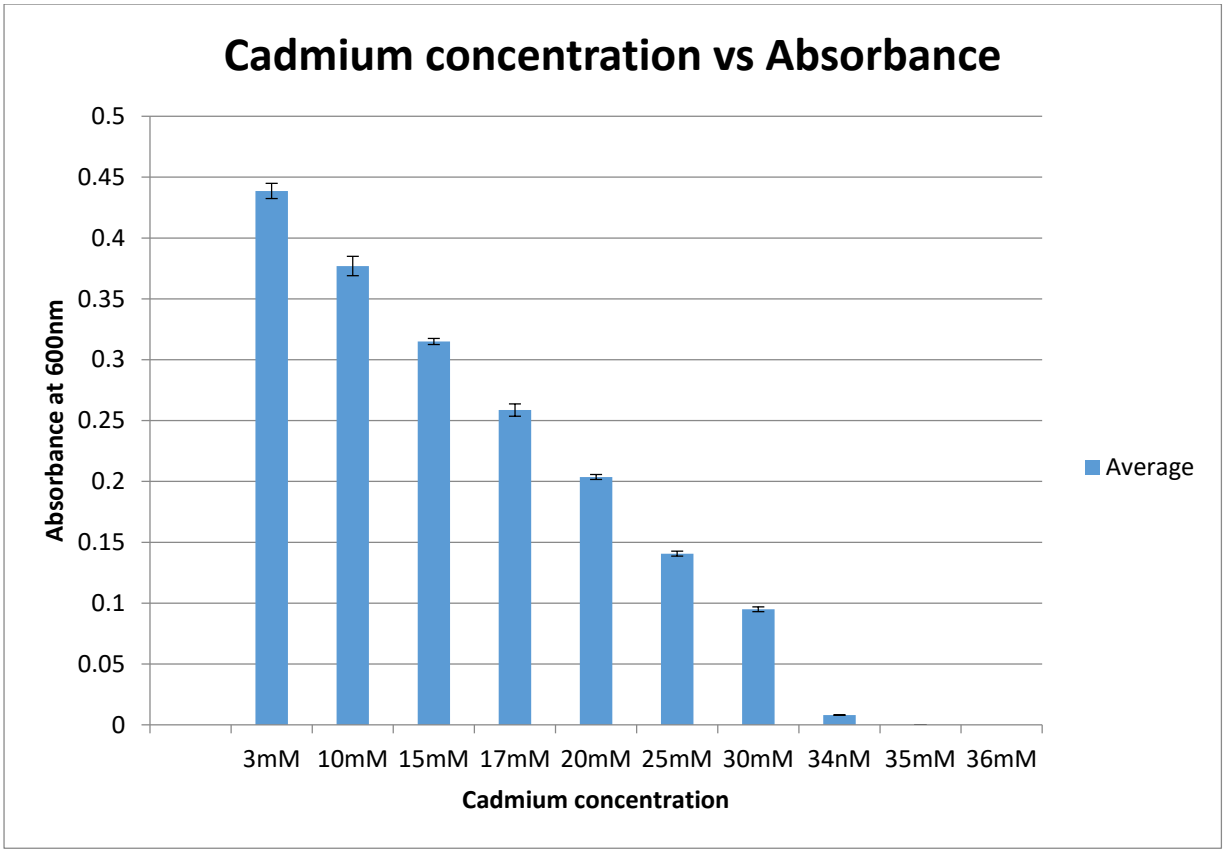

Figure 10: Minimum inhibitory concentration of the isolated sample C1

In the figure, it was observed that the isolated bacterium C1 has the ability to tolerate Cadmium concentration up to 34mM. So, it can be said that C1 was capable to demonstrate resistance till the Cadmium concentration of 34mM. However, from 35mM concentration, no growth of C1 was observed. Therefore, the Minimum Inhibitory Concentration for the C1 isolate is 35mM.

3.1.6 Minimum Inhibitory Concentration of Cadmium to inhibit the growth of Cadmium resistant bacteria C2

According to the procedure that is described in the section 2.10, the Minimum Inhibitory Concentration of the isolated sample B2 was determined and the obtained result is given below in the following table.

Table 16: MIC of Cadmium resistant organism B2 against different Cadmium concentration

Cd Concentration	Absorbance at 600nm Test Tube 1	Absorbance at 600nm Test Tube2	Absorbance at 600nm Test Tube3	Average	Standard Deviation
5mM	0.055	0.061	0.059	0.05833333	0.00305505
10mM	0.046	0.044	0.039	0.043	0.00360555
15mM	0.026	0.029	0.024	0.02633333	0.00251661
20mM	0.005	0.009	0.006	0.00666667	0.00208167
22mM	0.001	0.003	0	0.00133333	0.00152753
23mM	0	0	0	0	0
24nM	0	0	0	0	0

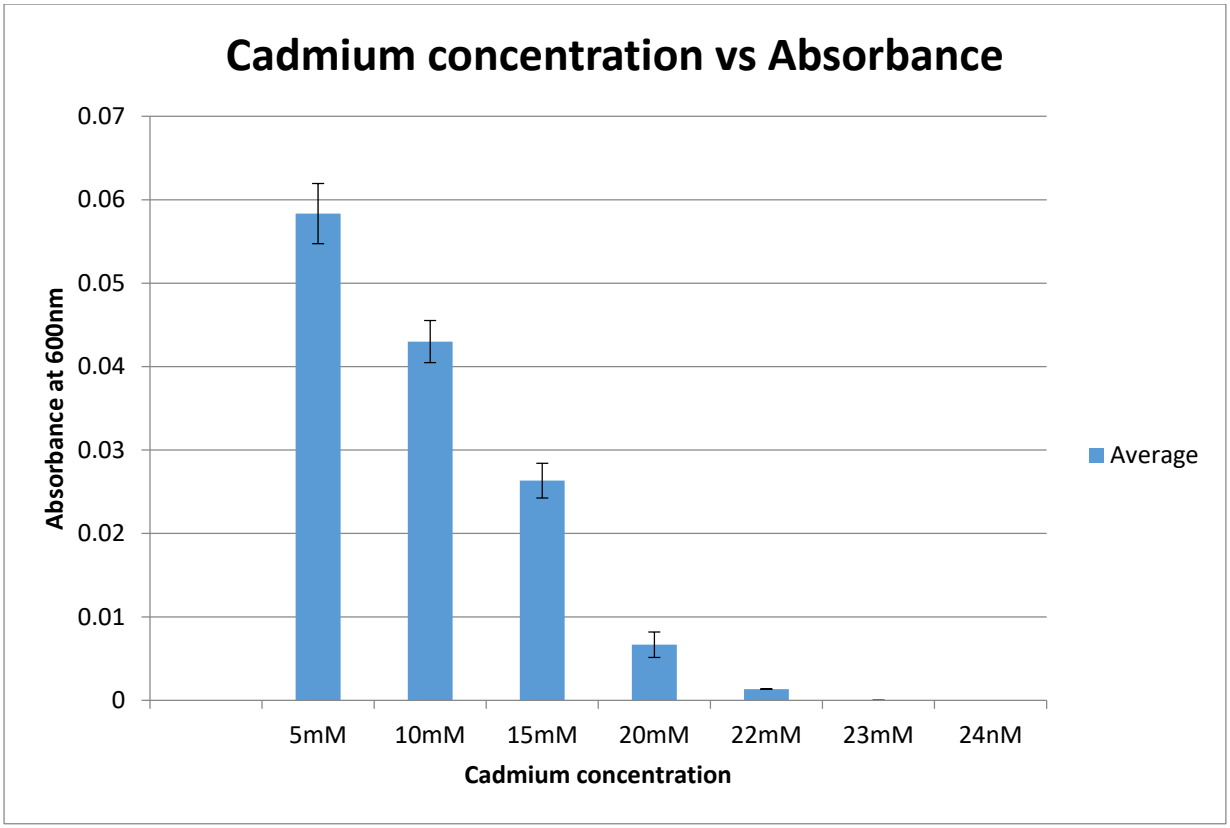

Figure 11: Minimum inhibitory concentration of the isolated sample C2

In the figure, it was observed that the isolated bacterium C2 has the ability to tolerate Cadmium concentration up to 22mM. So, it can be said that C2 was capable to demonstrate resistance till the Cadmium concentration of 34mM. However, from 23mM concentration, no growth of C2 was observed. Therefore, the Minimum Inhibitory Concentration for the C2 isolate is 23mM.

3.1.7 Minimum Inhibitory Concentration of Cadmium to inhibit the growth of Cadmium resistant bacteria D1

According to the procedure that is described in the section 2.10, the Minimum Inhibitory Concentration of the isolated sample D1 was determined and the obtained result is given below in the following table.

Table 17: MIC of Cadmium resistant organism D1 against different Cadmium concentration

Cd Concentration	Absorbance at 600nm Test Tube 1	Absorbance at 600nm Test Tube2	Absorbance at 600nm Test Tube3	Average	Standard Deviation
3mM	0.189	0.187	0.191	0.189	0.002
5mM	0.142	0.149	0.147	0.146	0.003606
7mM	0.119	0.117	0.12	0.118667	0.001528
10mM	0.085	0.088	0.08	0.084333	0.004041
15mM	0.071	0.079	0.075	0.075	0.004
17mM	0.055	0.049	0.048	0.050667	0.003786
20mM	0.029	0.028	0.033	0.03	0.002646
25nM	0.013	0.011	0.018	0.014	0.003606
34mM	0.002	0.003	0.007	0.004	0.002646
35mM	0	0	0	0	0
36mM	0	0	0	0	0

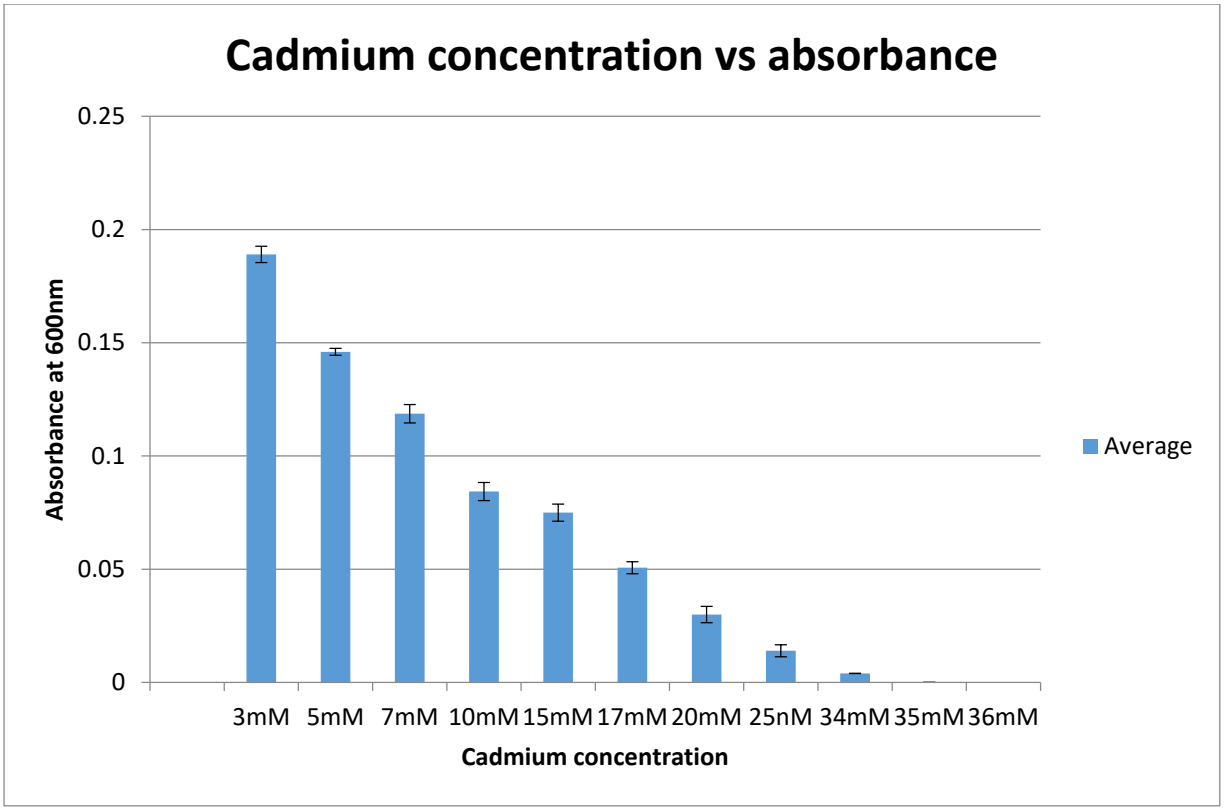

Figure 12: Minimum inhibitory concentration of the isolated sample D1

In the figure, it was observed that the isolated bacterium DI has the ability to tolerate Cadmium concentration up to 34mM. So, it can be said that D1 was capable to demonstrate resistance till the Cadmium concentration of 34mM. However, from 35mM concentration, no growth of D1 was observed. Therefore, the Minimum Inhibitory Concentration for the D1 isolate is 35mM.

3.1.8 Minimum Inhibitory Concentration of Cadmium to inhibit the growth of Cadmium resistant bacteria D2

According to the procedure that is described in the section 2.10, the Minimum Inhibitory Concentration of the isolated sample D2 was determined and the obtained result is given below in the following table.

Table 18: MIC of Cadmium resistant organism D2 against different Cadmium concentration

Cd Concentration	Absorbance at 600nm Test Tube 1	Absorbance at 600nm Test Tube2	Absorbance at 600nm Test Tube3	Average	Standard Deviation
3mM	0.163	0.169	0.166	0.166	0.003
5mM	0.147	0.149	,143	0.148	0.001414
7mM	0.127	0.122	0.129	0.126	0.003606
10mM	0.082	0.089	0.09	0.087	0.004359
15mM	0.056	0.059	0.049	0.054667	0.005132
17mM	0.017	0.02	0.019	0.018667	0.001528
20mM	0.003	0.006	0.006	0.005	0.001732
21nM	0	0	0	0	0
22mM	0	0	0	0	0

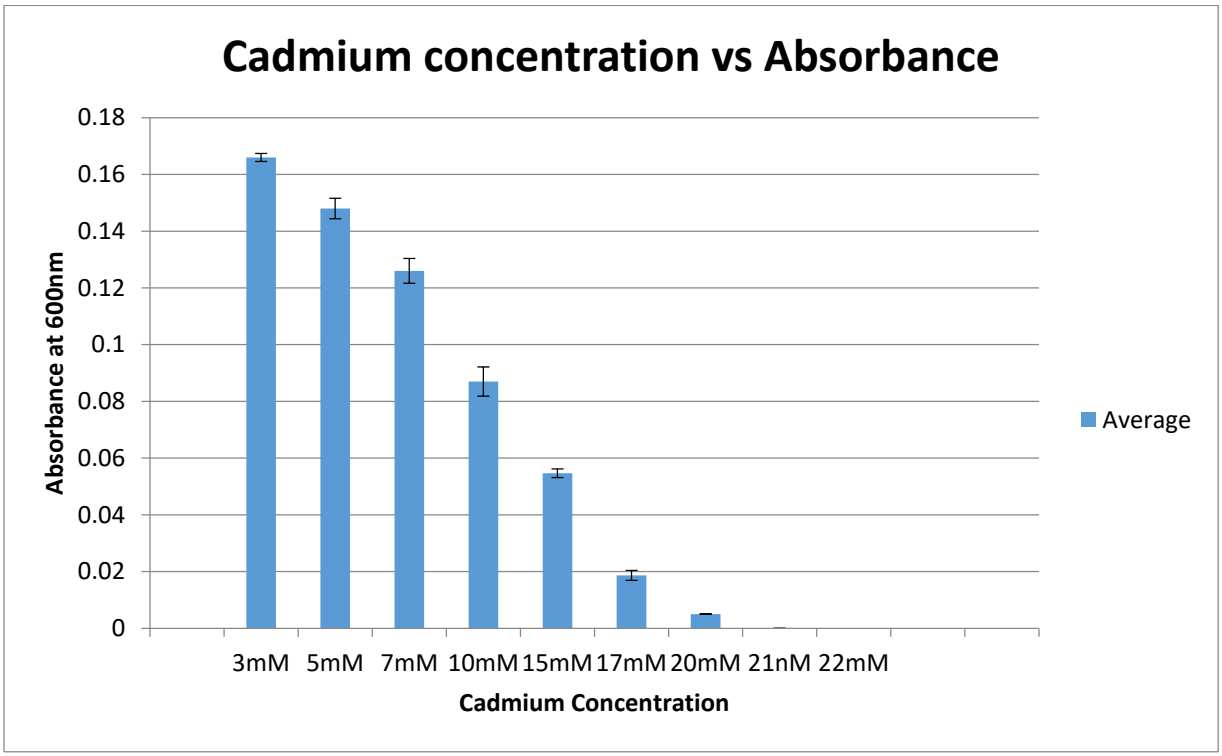

Figure 13: Minimum inhibitory concentration of the isolated sample D2

In the figure, it was observed that the isolated bacterium D2 has the ability to tolerate Cadmium concentration up to 20mM. So, it can be said that D2 was capable to demonstrate resistance till the Cadmium concentration of 20mM. However, from 21mM concentration, no growth of D2 was observed. Therefore, the Minimum Inhibitory Concentration for the D2 isolate is 21mM.

3.1.9 Minimum Inhibitory Concentration of Cadmium to inhibit the growth of Cadmium resistant bacteria E1

According to the procedure that is described in the section 2.10, the Minimum Inhibitory Concentration of the isolated sample E1 was determined and the obtained result is given below in the following table.

Table 17: MIC of Cadmium resistant organism E1 against different Cadmium concentration

Cd Concentration	Absorbance at 600nm Test Tube 1	Absorbance at 600nm Test Tube2	Absorbance at 600nm Test Tube3	Average	Standard Deviation
5mM	0.044	0.046	0.046	0.04533333	0.0011547
7mM	0.032	0.034	0.029	0.03166667	0.00251661
10mM	0.02	0.024	0.027	0.02366667	0.00351188
13mM	0.012	0.015	0.013	0.01333333	0.00152753
17mM	0.012	0.009	0.008	0.00966667	0.00208167
20mM	0.003	0.003	0.004	0.00333333	0.00057735
24mM	0.001	0.001	0.003	0.00166667	0.0011547
25nM	0	0	0	0	0
26mM	0	0	0	0	0

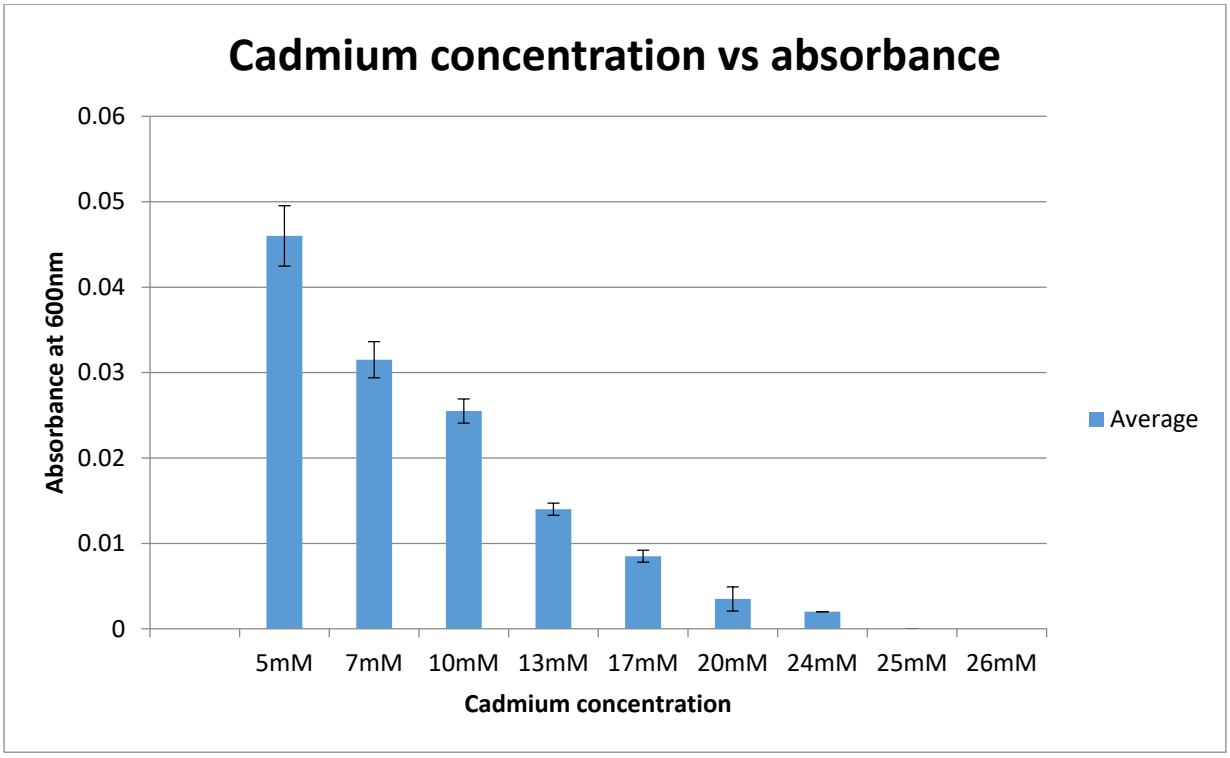

Figure 14: Minimum inhibitory concentration of the isolated sample E1

In the figure, it was observed that the isolated bacterium E1 has the ability to tolerate Cadmium concentration up to 24mM. So, it can be said that E1 was capable to demonstrate resistance till the Cadmium concentration of 24mM. However, from 25mM concentration, no growth of E1 was observed. Therefore, the Minimum Inhibitory Concentration for the E1 isolate is 25mM.

3.1.10 Minimum Inhibitory Concentration of Cadmium to inhibit the growth of Cadmium resistant bacteria E2

According to the procedure that is described in the section 2.10, the Minimum Inhibitory Concentration of the isolated sample E2 was determined and the obtained result is given below in the following table.

Table 18: MIC of Cadmium resistant organism E2 against different Cadmium concentration

Cd Concentration	Absorbance at 600nm Test Tube 1	Absorbance at 600nm Test Tube2	Absorbance at 600nm Test Tube3	Average	Standard Deviation
5mM	0.027	0.024	0.024	0.025	0.00173205
7mM	0.018	0.015	0.016	0.01633333	0.00152753
10mM	0.009	0.01	0.01	0.00966667	0.00057735
13mM	0.002	0.003	0.003	0.00266667	0.00057735
14mM	0	0	0	0	0
15mM	0	0	0	0	0

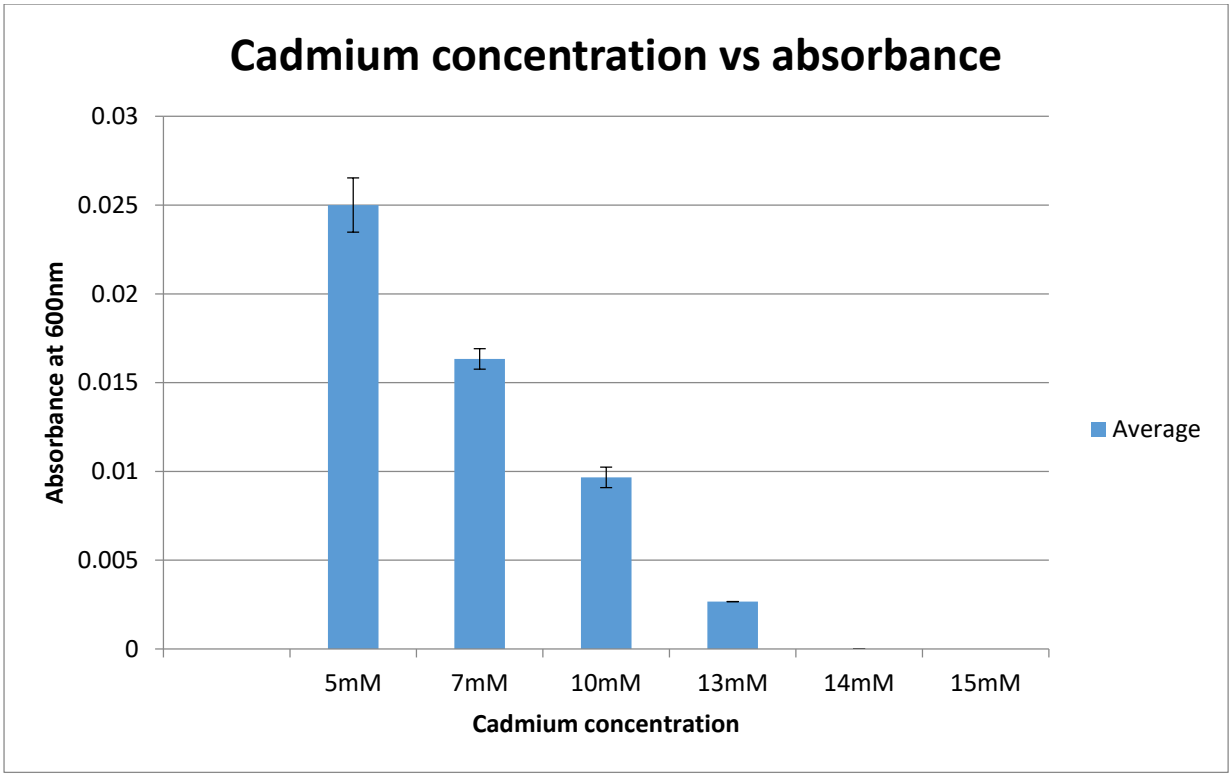

Figure 15: Minimum inhibitory concentration of the isolated sample E2

In the figure, it was observed that the isolated bacterium E2 has the ability to tolerate Cadmium concentration up to 13mM. So, it can be said that E2 was capable to demonstrate resistance till the Cadmium concentration of 13mM. However, from 14mM concentration, no growth of E2 was observed. Therefore, the Minimum Inhibitory Concentration for the E2 isolate is 14mM.

3.1.11 Minimum Inhibitory Concentration of Cadmium to inhibit the growth of Cadmium resistant bacteria F1

According to the procedure that is described in the section 2.10, the Minimum Inhibitory Concentration of the isolated sample D2 was determined and the obtained result is given below in the following table.

Table 19: MIC of Cadmium resistant organism F1 against different Cadmium concentration

Cd Concentration	Absorbance at 600nm Test Tube 1	Absorbance at 600nm Test Tube2	Absorbance at 600nm Test Tube3	Average	Standard Deviation
5mM	0.005	0.006	0.005	0.00533333	0.00057735
7mM	0.003	0.003	0.002	0.00266667	0.00057735
9mM	0.001	0.002	0.001	0.00133333	0.00057735
10mM	0	0	0	0	0
11mM	0	0	0	0	0

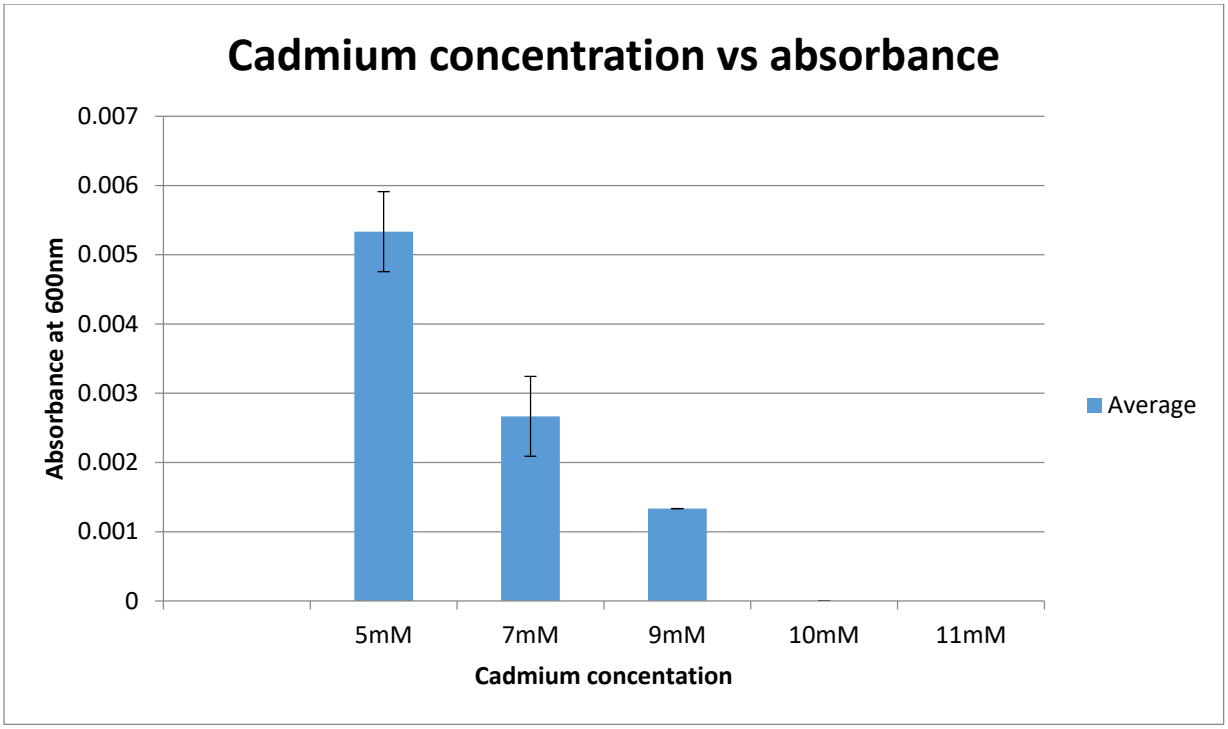

Figure 16: Minimum inhibitory concentration of the isolated sample F1

In the figure, it was observed that the isolated bacterium F1 has the ability to tolerate Cadmium concentration up to 9mM. So, it can be said that F1 was capable to demonstrate resistance till the Cadmium concentration of 9mM. However, from 10mM concentration, no growth of F1 was observed. Therefore, the Minimum Inhibitory Concentration for the F1 isolate is 10mM.

3.1.12 Minimum Inhibitory Concentration of Cadmium to inhibit the growth of Cadmium resistant bacteria F2

According to the procedure that is described in the section 2.10, the Minimum Inhibitory Concentration of the isolated sample F2 was determined and the obtained result is given below in the following table.

Table 20: MIC of Cadmium resistant organism F2 against different Cadmium concentration

Cd Concentration	Absorbance at 600nm Test Tube 1	Absorbance at 600nm Test Tube2	Absorbance at 600nm Test Tube3	Average	Standard Deviation
5mM	0.009	0.011	0.01	0.01	0.001
7mM	0.007	0.008	0.008	0.00766667	0.00057735
10mM	0.006	0.007	0.006	0.00633333	0.00057735
13mM	0.004	0.004	0.005	0.00433333	0.00057735
15mM	0.003	0.004	0.003	0.00333333	0.00057735
20mM	0.002	0.002	0.003	0.00233333	0.00057735
24mM	0	0.001	0.002	0.001	0.001
25mM	0	0	0	0	0
26mM	0	0	0	0	0

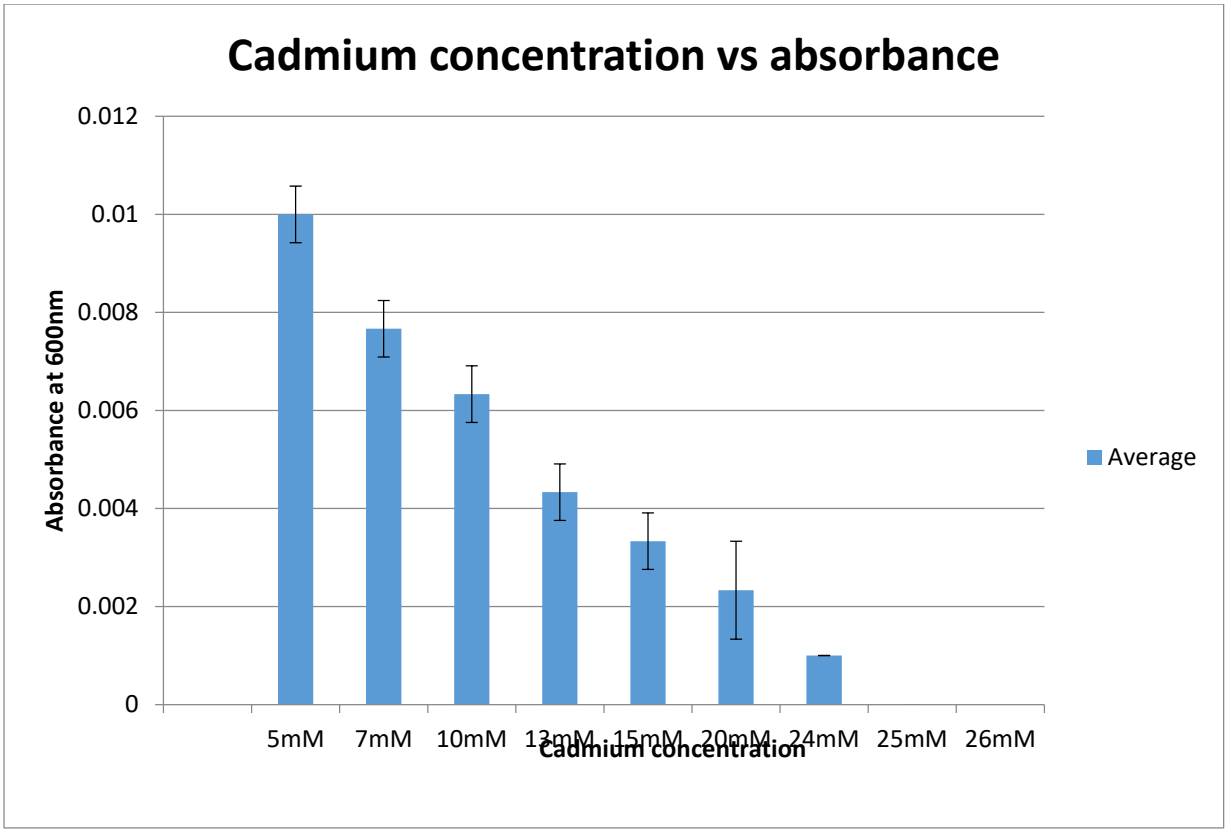

Figure 17: Minimum inhibitory concentration of the isolated sample F2

In the figure, it was observed that the isolated bacterium F2 has the ability to tolerate Cadmium concentration up to 24mM. So, it can be said that F2 was capable to demonstrate resistance till the Cadmium concentration of 24mM. However, from 25mM concentration, no growth of F2 was observed. Therefore, the Minimum Inhibitory Concentration for the F2 isolate is 25mM.

3.1.13 Minimum Inhibitory Concentration of Cadmium to inhibit the growth of Cadmium resistant bacteria G1

According to the procedure that is described in the section 2.10, the Minimum Inhibitory Concentration of the isolated sample G1 was determined and the obtained result is given below in the following table.

Table 21: MIC of Cadmium resistant organism G1 against different Cadmium concentration

Cd Concentration	Absorbance at 600nm Test Tube 1	Absorbance at 600nm Test Tube2	Absorbance at 600nm Test Tube3	Average	Standard Deviation
5mM	0.009	0.008	0.009	0.00866667	0.00057735
7mM	0.007	0.007	0.008	0.00733333	0.00057735
10mM	0.006	0.005	0.005	0.00533333	0.00057735
13mM	0.004	0.004	0.003	0.00366667	0.00057735
14mM	0.001	0	0.001	0.00066667	0.00057735
15mM	0	0	0	0	0
16mM	0	0	0	0	0

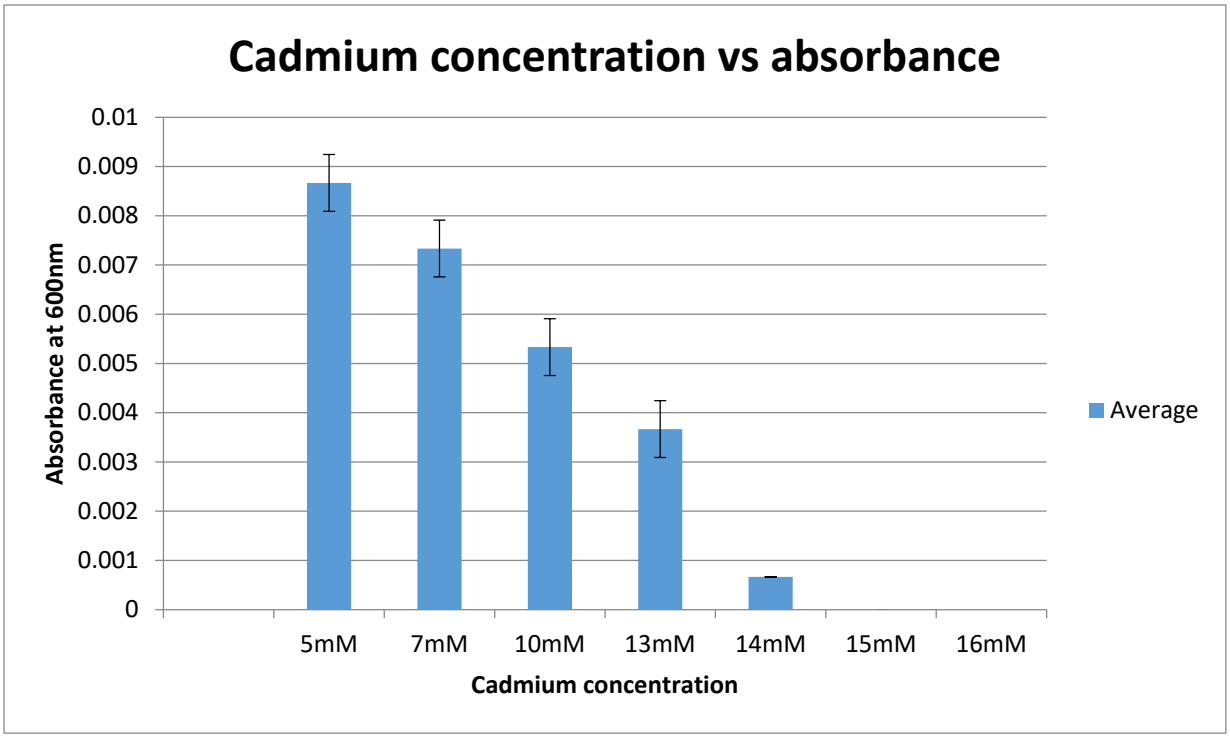

Figure 18: Minimum inhibitory concentration of the isolated sample G1

In the figure, it was observed that the isolated bacterium G1 has the ability to tolerate Cadmium concentration up to 14mM. So, it can be said that G1 was capable to demonstrate resistance till the Cadmium concentration of 14mM. However, from 15mM concentration, no growth of G1 was observed. Therefore, the Minimum Inhibitory Concentration for the G1 isolate is 15mM.

3.1.14 Minimum Inhibitory Concentration of Cadmium to inhibit the growth of Cadmium resistant bacteria G2

According to the procedure that is described in the section 2.10, the Minimum Inhibitory Concentration of the isolated sample G2 was determined and the obtained result is given below in the following table.

Table 22: MIC of Cadmium resistant organism G2 against different Cadmium concentration

Cd Concentration	Absorbance at 600nm Test Tube 1	Absorbance at 600nm Test Tube2	Absorbance at 600nm Test Tube3	Average	Standard Deviation
5mM	0.011	0.01	0.01	0.01033333	0.00057735
7mM	0.009	0.009	0.008	0.00866667	0.00057735
10mM	0.007	0.008	0.007	0.00733333	0.00057735
15mM	0.005	0.005	0.006	0.00533333	0.00057735
17mM	0.004	0.003	0.003	0.00333333	0.00057735
18mM	0	0	0	0	0
19mM	0	0	0	0	0

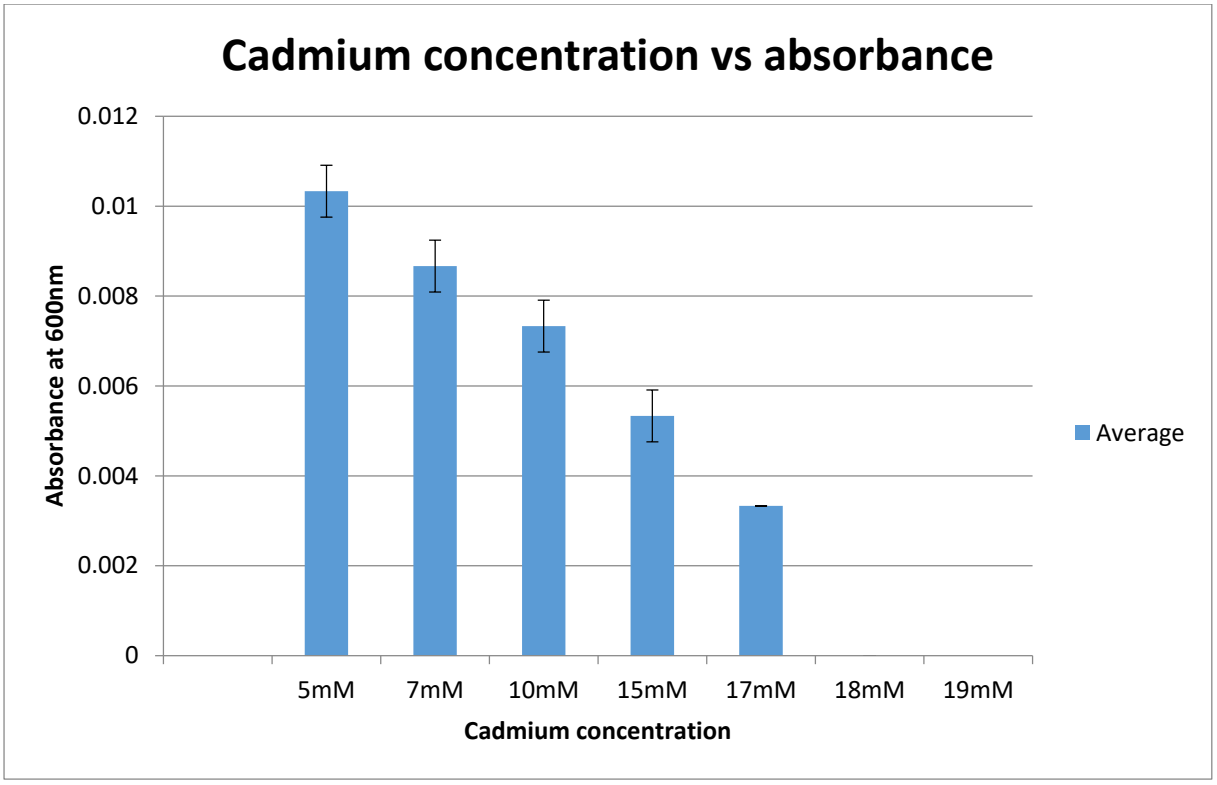

Figure 19: Minimum inhibitory concentration of the isolated sample G2

In the figure, it was observed that the isolated bacterium G2 has the ability to tolerate Cadmium concentration up to 17mM. So, it can be said that G2 was capable to demonstrate resistance till the Cadmium concentration of 17mM. However, from 18mM concentration, no growth of G2 was observed. Therefore, the Minimum Inhibitory Concentration for the G2 isolate is 18mM.

3.2 Discussion

Cadmium is one of the toxic heavy metals, which is abundantly exposed and spread out in the environment following several ways for example; from the dyeing industries and the tannery industries. In present it has become a threat to the entire human life and also to the other livestock. Because of the carcinogenic nature of cadmium, it has now become one of the greatest concerns of people to find out the proper and valid solution to this issue. The purpose

of this study was to find out the minimum inhibitory concentration of cadmium to different bacterial strains that were collected from the environment.

For the purpose of this experiment, the needed sample was collected from the pargandaria and showarighat area which is located near Buriganga River. Most of the tannery industries of Bangladesh are situated in these areas. The waste of these industries is released in the Buriganga river. As a result, this river contains a very large amount of heavy metals. Cadmium is one of them. We have collected our sample from these areas and then purification and isolation was done as well. Then the experiment of MIC test of 14 different bacterial strains was done by following the standard protocol.

In minimum inhibitory concentration testing of the bacterial strains, it was observed that the isolate A1 was capable of tolerating up to 13mM concentration of Cadmium. At a higher concentration than this, no absorbance was found. So the MIC of isolate A1 is 14mM. From the result, it can also be said that these bacterial strains can survive up to 13mM concentration of cadmium. On the other hand, isolate A2 was capable of tolerating up to 17mM concentration of Cadmium. At a higher concentration than this, no absorbance was found. So the MIC of isolate A2 is 18mM. From the result, it can also be said that these bacterial strains can survive up to 17mM concentration of cadmium.

The isolate B1 was capable of tolerating up to 27mM concentration of Cadmium. At a higher concentration than this, no absorbance was found. So the MIC of isolate B1 is 28mM. From the result, it can also be said that these bacterial strains can survive up to 27mM concentration of cadmium. On the other hand, the isolate B2 was capable of tolerating up to 26mM concentration of Cadmium. At a higher concentration than this, no absorbance was found. So the MIC of isolate B2 is 27mM. From the result, it can also be said that these bacterial strains can survive up to 26mM concentration of cadmium.

In case of the isolate C1, it was capable of tolerating up to 34mM concentration of Cadmium. At a higher concentration than this, no absorbance was found. So the MIC of isolate C1 is 35mM. From the result, it can also be said that these bacterial strains can survive up to 34mM concentration of cadmium. On the contrary, isolate C2 was capable of tolerating up to 22mM concentration of Cadmium. At a higher concentration than this, no absorbance was found. So the MIC of isolate C2 is 23mM. From the result, it can also be said that these bacterial strains can survive up to 22mM concentration of cadmium.

The isolate D1 was capable of tolerating up to 34mM concentration of Cadmium. At a higher concentration than this, no absorbance was found. So the MIC of isolate D1 is 35mM. From the result, it can also be said that these bacterial strains can survive up to 34mM concentration of cadmium. In case of the isolate D2, it was capable of tolerating up to 20mM concentration of Cadmium. At a higher concentration than this, no absorbance was found. So the MIC of isolate D2 is 21mM. From the result, it can also be said that these bacterial strains can survive up to 20mM concentration of Cadmium.

In the MIC test, it was found that the isolate E1 was capable of tolerating up to 24mM concentration of Cadmium. At a higher concentration than this, no absorbance was found. So the MIC of isolate E1 is 25mM. From the result, it can also be said that these bacterial strains can survive up to 24mM concentration of Cadmium. On the other hand**,** the isolate E2 was capable of tolerating up to 13mM concentration of Cadmium. At a higher concentration than this, no absorbance was found. So the MIC of isolate E2 is 14mM. From the result, it can also be said that these bacterial strains can survive up to 13mM concentration of Cadmium.

The isolate F1 was capable of tolerating up to 9mM concentration of Cadmium. At a higher concentration than this, no absorbance was found. So the MIC of isolate F1 is 10mM. From the result, it can also be said that these bacterial strains can survive up to 9mM concentration

of Cadmium. On the other hand, it was found that the isolate F2 was capable of tolerating up to 24mM concentration of Cadmium. At a higher concentration than this, no absorbance was found. So the MIC of isolate A1 is 25mM. From the result, it can also be said that these bacterial strains can survive up to 24mM concentration of Cadmium.

According to the result, the isolate G1 was capable of tolerating up to 14mM concentration of Cadmium. At a higher concentration than this, no absorbance was found. So the MIC of isolate G1 is 15mM. From the result, it can also be said that these bacterial strains can survive up to 14mM concentration of cadmium. On the other hand, the isolate G2 was capable of tolerating up to 17mM concentration of Cadmium. At a higher concentration than this, no absorbance was found. So the MIC of isolate G2 is 18mM. From the result, it can also be said that these bacterial strains can survive up to 17mM concentration of cadmium.

The MIC of cadmium to 14 different isolated bacterial strains are mentioned in table 23 in ascending order.

Table 23: MIC of cadmium to different bacterial strains

Name of the bacterial strain	MIC of Cadmium
C1	35mM
D1	35mM
B1	28mM
B2	27mM
F2	25mM
E1	25mM

C2	23mM
D2	21mM
G2	18mM
A2	18Mm
G1	15Mm
A1	14mM
E2	14mM
F1	10mM

It is observed that, C1 and D1 both the bacterial strains can outstand the highest MIC of cadmium compared to the other 13 isolated bacterial strains, which is 34mM. F1 can outstand the least concentration of Cadmium which is 10mM. This is the lowest among all the bacterial strains that we have worked with.

Chapter 4

CONCLUSION

4.1 Conclusion

After all the studies, all the MIC of Cadmium to 14 different bacterial strains was determined. The MIC of Cadmium to A1, A2, B1, B2, C1, C2, D1, D2, E1, E2, F1, F2, G1, G2 was found to be 14mM, 18mM, 28mM, 27Mm, 35mM, 23mM, 35mM, 21mM, 25mM, 14mM, 10mM, 25mM, 15mM, 18mM respectively. C1 and D1 both were found to have the highest MIC, which is 34mM. F1 was found to having the least MIC of Cadmium which is 10mM.

4.2 Future works

From this study, in future, studies can be done to find out the Cadmium reduction profile of these bacteria and it can be co-related with antibiotic resistance profile and Cadmium reduction assay. These bacterial strains can be used in future in heavy metals reduction purpose. Moreover, to get more information’s about these bacteria, plasmid analysis can be done.

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