Biotoxicological Investigations of Heavy metals

[Cr (VI) and As (III)] on different Bacteria

Plants and Animals

By

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A thesis submitted in partial fulfillment of the requirements for the Degree of

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Dedication

Every challenging work needs self-effort as well as guidance from elders.

especially those who are very close to our heart.

My humble effort I dedicate to my sweet and loving.

Father, Mother

And

Siblings.

Whose affection, love, encouragement and prayers of day and night make me.

able to get such success and honor.

And my constant source of guidance, strength, motivation

Prof. Dr. Naeem Ali

Declaration

The material and information contained in this thesis is my original work. I have not previously presented any part of this work elsewhere to any other degree.

Maria Rauf

Certificate

This report, submitted by Ms. Maria Rauf to the Department of Microbiology, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan, is accepted in its present form as satisfying the thesis requirement for the degree of Master of Philosophy in Microbiology.

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List of Acronyms

Acronym	Word
E.coli	*Escherichia coli*
B.sub	*Bacilus Subtilis*
S.L	*Solanum lycopersicum*
E.S	*Eruca Sativa*
A.salina	Artemia Salina
As (III)	Trivalent Arsenic
Cr (VI)	*Hexavalent chromium*
HMs	Heavy metals
FTIR	Fourier transformed infrared spctrophotometry
UV-VIS	Ultraviolet Visible
IARC	International agency for research on cancer
WHO	World health organization
EPSs	Exopolysaccharides
ROS	Reactive oxygen specie
MRP	Multi-Drug Reselient protein
L.B	Luria Broth
O.D	Optical Density
DPC	Diphenyl carbazide
LDH	Lactate dehydrogenase
PBS	Phosphate buffer saline
K_2Cr2O_7	Potassium dichromate
$NaAsO_2$	Sodium Arsenite
HCL	Hydrochloric acid

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All praises to **Almighty Allah,** The Light of Heavens and Earths, The One Who puts good thoughts in one's mind, turn them into determinations and then makes the way towards their fulfillments showering all His Blessings throughout the journey.

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Abstract

Natural substances known as heavy metals exhibit huge atomic masses with densities which are at least five times higher than those of water. Their widespread dispersion in ecosyplumules as a result of their diverse commercial, residential, agricultural, medical, and technical projects has sparked worries about their possible environmental and social health repercussions. Nevertheless, their inappropriate utilization can have a detrimental effect on agricultural productivity and soil microorganisms, destructive to marine life as well as cause environmental problems. taking into account these considerations, an in vitro investigation was conducted to assess the impact of heavy metals, Cr (VI) and As (III), at suggested, lower, and higher concentration levels on the growth pattern and membrane integrity of isolated microbial specimens (*Escherichia coli* and *Bacillus subtilis*). Additionally, their harmful effects were examined in relation to the development and germination of plant seeds (*Solanum lycopersicum* and *Eruca sativa*), and animal cells (*Artemia salina*). The development of examined bacteria, plants and animal cells subjected to both heavy metals with concentrations beyond the suggested dose has shown a concentration-dependent almost equal sequential drop in cells in treatment with both heavy metals, according to the research findings. However, in comparison the bacterial cells showed variable sensitivity to both heavy metals, (as both are toxic) but *E. coli* showed greater sensitivity to As (III), while *B. subtilis* exhibit higher sensitivity to Cr (VI). Similarly in the case of plants both heavy metals exhibit almost equal toxic behavior to the selected plant seeds. On the other hand, this case was different in exposure to Artemia salina which demonstrated higher sensitivity to As (III). Moreover, the spectrophotometric and FTIR (Fourier transform infrared) outcomes demonstrated that, in a shake flask experiment, the selected species of *B. subtilis* and *E. coli* displayed 98 and 83% bioremoval of Cr (VI) and 98% bioremoval of As (III) in 48 hours. In summary, the current findings provide valuable insights regarding the mechanical perspective of heavy metal-induced cellular toxicity toward bacteria, plants, and animals. Additionally, they demonstrate the possibility for bacterial cells to bio transform these metals in a way that mitigates their detrimental repercussions.

Introduction

Industrialization has been increasing with the continuous development in urbanization and population with the increasing requirements of industrial scale products and modern agriculture rapidly. Meanwhile, this modernization also causes great environmental damage due to the discharge of highly contaminated effluent along with the harmful chemicals from industries which then became the part of drinking water system, contaminate the soil, and damage marine ecosystem as well(Kandhol et al., 2022). This wastewater contains a large variety of toxic heavy metals which include (pb, As, Cu, Cd, Hg, Zn, Co, Ni, Fe, Cr, etc.). they show toxicity by varying degrees of growth inhibition, even at extremely low concentrations(Vardhan, Kumar, & Panda, 2019). Heavy metal overabundance in soil and aquatic habitats can have detrimental cytotoxic effects on microbes and phytotoxic effects, including stunted growth, disturbed photosynthesis, biomass reduction, and inadequate nutrient absorption(Manu, Onete, & Băncilă, 2018). There are significant hazards to the current flora and fauna due to environmental pollution caused by heavy metal contamination, including mercury (Hg), lead (Pb), chromium (Cr), Arsenic (As) and others(Witkowska, Słowik, & Chilicka, 2021).

Depending on the properties of the soil (pH, type, salinity, etc.), some soil-dwelling microorganisms have the ability to initiate and grow a range of metal mobilization or immobilization processes (such as biosorption, bioprecipitation(Hlihor et al., 2014). The diversity and abundance of microorganisms can be dramatically altered by heavy metals(Zhang et al., 2019). Heavy metal sensitivity varies across various categories of bacteria(Brito et al., 2015). When microbial diversity and community structure are taken into account in its whole, the function of the microbial community can be utilized as an indicator to reflect heavy metal pollution(Tang et al., 2019).However, Plants that are able to absorb large levels of metal ions from heavy metal-contaminated soils can eventually enter the food chain and have an impact on human health(Jutsz & Gnida, 2015). Similarly, Therefore, the use of microbes, plants, or other biological system to clean up contaminated soils under regulated conditions and within the bounds of their tolerance for heavy metals, continues to be a challenge for researchers and regulatory bodies(Vardhan et al., 2019),(Sobariu et al., 2017).

Of these pollutants, arsenic (As) and chromium (Cr) have drawn particular attention due to their quick environmental buildup, migration, and acute toxicity(Pal et al., 2017). Their toxicity extends to microbes, aquatic organisms as well as plants, and their reaction to any stress caused by heavy metals is contingent upon the kind, concentration, and speciation of the heavy metals as well as environmental conditions and the species of the organisms(Alessandrello & Vullo, 2018). When heavy metal concentrations in the environment exceed threshold limits, especially those of chromium (Cr) and Arsenic (As), the plants are severely poisoned and their physiological, biochemical, and molecular characteristics are frequently negatively impacted. Due to their recalcitrance, their long-term existence and retention in the environment causes delays in the germination of seeds, an overall reduction in radicle growth and biomass, compromised photosynthetic parameters, and ultimately, the death of the plant(Saud et al., 2022). In aquatic ecosystem these heavy metals also adversely affect the growth rate well as the survival of marine flora and fauna. (Trompeta, Preiss, Ben-Ami, Benayahu, & Charitidis, 2019). and In humans, Arsenic increases the risk of skin damage, circulatory system changes, and cancer. Cr, on the other hand, has the potential to cause allergy dermatitis and cancer(Ballav, Maity, & Mishra, 2012).

Cr typically appears in water bodies as oxygen-containing trivalent (Cr (III)) or hexavalent (Cr (VI)) anions. throughout highly oxidizing conditions, Cr(VI) is found as oxyanions ($Cr_2O_7^{2-}$, $HCrO_4^{2-}$, and CrO_4^{2-}) throughout a wide pH range of 2.0 to 14.0(Choppala, Bolan, & Park, 2013). On the other hand, in moderately oxidizing and reduced conditions, Cr(III) often occurs as a somewhat soluble hydroxide Cr(OH)3 at pH = 8.2–9.4 or a soluble oxyanion $Cr(OH)4^-$ at pH > 12.0(Choppala et al., 2013). The most noxious variant of Cr is Cr (VI) wherever It is well known that Cr (III) is required to maintain the glucose metabolism of proteins and lipids. Moreover, Cr(III) can maintain the configuration of DNA, RNA, and proteins at their tertiary levels(Pavel, Sobariu, Fertu, Statescu, & Gavrilescu, 2013). In many industries, including metallurgy, leather tanning, pigment, mining, electroplating, corrosion mitigation, and electronic and electrical devices, Cr is utilized extensively(S. Zhou et al., 2014). Despite being carcinogenic and mutagenic to most organisms, even at low concentrations, Cr(VI) and As (III) are also considered as

Group "A" human carcinogens(J. Wang et al., 2017). Therefore, changing the valence states of As and Cr (converting As (III) to As(V) and Cr (VI) to Cr(III)) is a promising way to lessen the adverse environmental impacts caused by both As and Cr.

Likewise, As(III) and As(V) are the two major categories of arsenic that are known to exist in aquatic environments(Wenzel, 2013). As(V) is the variant that is thermodynamically stable in oxygenated aquatic habitats , whereas As(III) is generally stable in mildly reducing circumstances, broadly ranging from −0.2 V at pH 9.0 to +0.3 V at pH 4.0 oxidation potentials(Sarkar & Paul, 2016). The main causes of contamination related to As are mining operations and herbicides containing As(M. F. Ahmed, Mokhtar, & Alam, 2021). The World Health Organization considers arsenic to be an extremely dangerous carcinogen for humans, plants, microorganisms and marine animals a well(Paula, Froes-Silva, & Ciminelli, 2012). The uncoupling of oxidative phosphorylation and inhibition of mitochondrial enzymes are indicative of As(III)'s harmful effects(Scott, Hatlelid, MacKenzie, & Carter, 1993). Hence, As(V) tends to be less mobile than As(III) and can be extracted from water more easily by adsorbing on a heterogeneous surface(Hassan, 2018). Although methylation was once thought to be a stage in detoxification, evidence currently suggests that the toxicity of As can be summarized as follows: monomethylarsonic acid (III) > As(III) > As(V) > dimethylarsinic acid (V) > monomethylarsonic acid (V)(Kile et al., 2011).

Here, in this research, investigations on the ecotoxicity of Cr (VI) and As (III) were conducted utilizing isolated bacterial strains of (*E. coli* and *B. subtilis.)* by elucidating their toxic effects on the growth of bacteria including their assessment on the membrane integrity of the bacteria and bioremoval of heavy metals. Additionally, the phytotoxicity of Cr (VI) and As (III) has been evaluated by examining how they affect the plants (*solanum lycopersicum* and *Eruca sativa)*'s ability to germinate seeds and reduction in growth rate of radicles, plumules, and dry biomass. Moreover, the ecotoxicological behavior of these metals were investigated on the marine species animal cell (*Artemia salina*), as *A. salina* is a commonly used model organism in toxicological investigations because of its short lifespan period, easy culture cultivation, high offspring production, availability of its cysts

at commercial scale, availability throughout the year, low cost, no need for feeding during the assay.

Aims and objectives

Aim

Evaluation of Biotoxicological assessment and comparative analysis of heavy metals, [Cr (VI) and As (III)], On the activity of different bacteria, (*E. coli* and *B. subtilis*) plants (*Solanum lycopersicum* and *Eruca sativa*) and animals (*Artemia salina*)

Objectives

1.To evaluate Bacterial growth by the action of heavy metals via spectrophotometric method

- To investigate detoxification of heavy metals by potential microbial strains via spectrophotometry,
- To evaluate bacterial membrane integrity via LDH assay,
- To demonstrate bacterial growth kinetics through effective concentration of heavy metals via spectrophotometric method
- To determine biosorption of heavy metals via microbial strains.

2. To elucidate Phyto toxicological analysis of heavy metals using plants i.e. *solanum lycopersicum* and *Eruca sativa*

- To determine seed germination rate
- To elucidate RADICLE length inhibition
- To check Plumule length inhibition
- To determine Dry biomass inhibition
- Biosorption of heavy metals by plants.

3. To analyze lethality associated with heavy metals to animal cells i.e. *Artemia salina*

Literature review

Over the past century, industrialization has rapidly expanded. As a result, it has raised demand for the reckless plundering of the natural assets of the planet, aggravating the global ecological and environment pollution issue(P. K. Gautam, Gautam, Banerjee, Chattopadhyaya, & Pandey, 2016). variety of contaminants, including inorganic ions, organic contaminants, isotopes of radioactive substances, organometallic substances, volatile contaminants, heavy metals and nanoparticles, have significantly contaminated the environment(Walker, Sibly, Hopkin, & Peakall, 2012). Heavily metallized particles, or HMs, are naturally occurring substances that can accumulate in the environment and in living things and constitute a health risk.

Heavy metal pollution is a major concern for the environment. One of the two reasons they are regarded as heavy metals is their elevated density or massive atomic weights. Currently, metallurgical chemical compounds and metalloids that are detrimental to the environment and human beings are referred to as "heavy metals." Certain metalloids and lighter metallic substances like aluminum, arsenic, and selenium are hazardous(Briffa, Sinagra, & Blundell, 2020). They have been referred to as heavy metals, nevertheless some of them—like the element gold—are usually not potentially hazardous(Tchounwou, Yedjou, Patlolla, & Sutton, 2012). The following is a list of heavy metals that are progressively more prevalent in daily life and have densities higher than 5 g/cm^3: Silver, Cadmium , Tin , Platinum , Gold, Mercury , Lead, Titanium , Vanadium, Chromium , Manganese , Iron, Cobalt , Nickel , Copper , Zinc, Arsenic , Molybdenum(Briffa et al., 2020).

2.1 Causes of heavy metal contamination into the environment

With the emergence of the Earth's surface, these metallic elements have existed naturally on the planet's crust. An impending explosion of metallic compounds in both the aquatic and terrestrial realms is the consequence of the startling growth in the consumption of heavy metals(P. K. Gautam et al., 2016). Anthropogenic activities are the primary causes of pollution in the ecosystem, and they mainly are responsible for heavy metal-related

contamination. These activities include mineral extraction, smelting, manufacturing plants, along with further metal-based sectors, as well as the leaching process of metals from multiple sources like landfills, waste dumps, drainage, livestock along with chicken manure, the runoff, automobiles, and road construction(P. K. Gautam et al., 2016; Masindi & Muedi, 2018). The supplementary factor contributing to heavy metal contamination in agriculture has been the Metallic intake from pesticides, herbicides, and other agricultural products. Additionally, natural processes including volcanic eruptions, metallic corrosion, vaporization of metal from soil and water, sedimentary re-suspension, eroding soil, and geological weathering may worsen the environmental damage caused by heavy metals(Tchounwou et al., 2012; Walker et al., 2012).

2.2 Characteristics of heavy metals

Metallic substances exhibit toxicological characteristics because they frequently establish covalent bonds. This characteristic has two main implications: firstly, it allows them to form covalent bonds with organic molecules(Gu et al., 2022). Therefore, when they attach to nonmetallic components of biological macromolecules, they can produce highly lipophilic ions and complexes that can have harmful effects(X.-F. Yang et al., 2022). Lipophilicity causes metalloids to behave differently from conventional ionic forms of the same element in terms of diffusion within the planet's biosphere and potentially dangerous reactions. The extremely lethal methylated versions of arsenic and tributyltin oxide are two examples of lyophilic chemicals. Lead and mercury are two examples of substances that interact to nonmetallic substances via attaching to the protein sulfhydryl groups(Masindi & Muedi, 2018). There are four potential routes whereby heavy metals might enter a human body: by means of consuming contaminated foods, breathing in polluted surroundings, drinking polluted water, or coming into touch with skin from manufacturing, dwellings, commercial, or agricultural settings(Fu & Xi, 2020).

Heavy Metals are not biodegradable and incapable of disintegrating(Mohammed Danouche, El Ghachtouli, & El Arroussi, 2021). By encasing the active component in a protein or storing them in intracellular granules that remain in an insoluble state that can be eliminated by the living being in its feces or retained for extended periods of time,

organisms are capable of detoxifying metallic ions(A. Kumar et al., 2021). Our system experience bioaccumulation of heavy metals when they are ingested or breathed in. They are categorized as hazardous as a result. Physiological and metabolic problems result from this bioaccumulation of metallic ions(Moiseenko & Gashkina, 2020). Certain heavy metals are referred to as essential components because they are needed for a number of biological and physiological activities that are vital to life. Yet if available in high concentrations, they may be harmful. Their extensive usage in the fields of harvesting, manufacturing, medical treatment, and other fields has caused them to be discharged throughout the earth's atmosphere, rivers, and landscapes(C. V. Raju et al., 2023).

2.3 Epidemiology of heavy metals

The hazardous effects of HM are global. Nevertheless, the frequency and severity of the toxic effects associated with specific heavy metals (HMs) differ depending on aspects such as geographical distribution, naturally occurring soil content, cultural practices, industry spot, regulatory actions to control contamination, health care institutions for determining HM toxic exposure, and genetic and nutritional state. Emission of a heavy metal into the surrounding environment, water, or soil may end up in its absorption by plants, crops, aquatic creatures, and livestock, which then completes the food web and reaches human beings(Joshi et al., 2023). Being exposed to heavy metals (HM) in workplaces as well as industrial settings, through ingesting or direct contact with the skin may trigger intoxication(N. H. Kim et al., 2015). Four out of the ten fundamental contaminants designated by the World Health Organization (WHO) are heavy metals(Chowdhury, 2022). Human consumption of HM-contaminated groundwater may contribute to chronic Heavy metal poisoning. An illustration would be the elevated levels of the arsenic in the groundwater in Bangladesh's neighbor and Bengal, India, where the content of arsenic in the drinking water is significantly higher than acceptable standards(M. M. Rahman et al., 2001).

There have been several instances of pandemic levels of heavy metal poisoning from time to time, which is the consequence of the relatively irresponsible discharge of hazardous industrial wastewater into the air, land, sea, and rivers. One famous instance involves a

gasoline company in California that dumped trash into the groundwater, leading to chromium (VI) poisoning of the area's groundwater supplies(Hausladen, Alexander-Ozinskas, McClain, & Fendorf, 2018). In Iraq, there was a mercury-related pandemic caused by eating cereals sprinkled with pesticides(Pallavi Sharma & Singh, 2016). Additionally, The deadly Minamata sickness, which was caused by the industrial leakage of mercury-methyl compounds into streams and seawater, poisoned and killed people in Japan(Budnik & Casteleyn, 2019). An additional instance from Japan involves the build-up of Cd in bones, leading to stiffness and ruptures, a condition known as "Itai-Itai (it hurts-it hurts) syndrome" (Aoshima, 2012). Epidemiological research has demonstrated a link between excessive exposure to heavy metals and long-term health issues such as carcinoma, type 2 diabetes, kidney ailments, neurodegenerative disorders, skin illnesses, respiratory disorders, and cardiovascular failure(Rehman, Fatima, Waheed, & Akash, 2018).

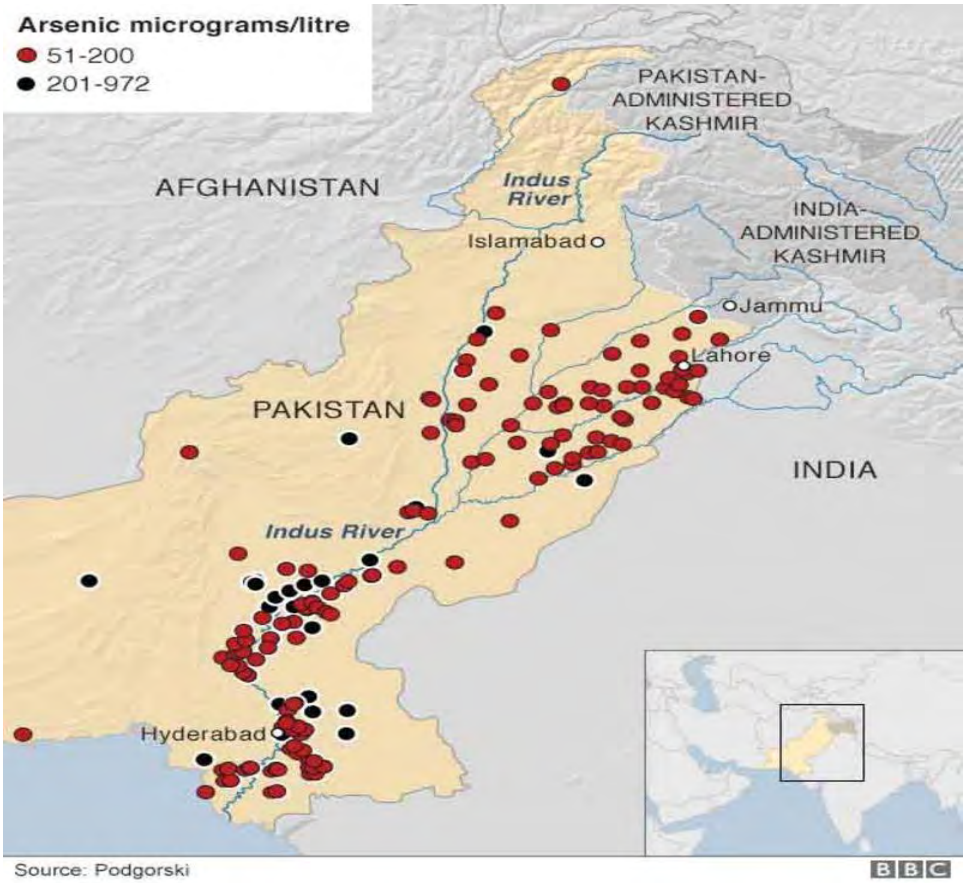


Figure : 2.1 A map illustrating the levels of arsenic in the water along the Indus valley in Pakistan (Grath, 2017)**.**

2.4 The toxicological effects of metals

It has been documented that heavy metals can impact organelles of the cell and components as well, including metabolic enzymes, lysosomes, cell membranes, the nuclei, and mitochondria. Reactive oxygen species (ROS) or free radicals exclusive to heavy metals lend themselves to cells more susceptible to oxidative stress(Wu et al., 2016). It has been discovered that metallic ions come into contact with nuclear proteins as well as DNA, triggering DNA damage that alters cell cycle progression and may cause apoptosis or cancer. There are two possible forms of damages: "direct" and "indirect" liabilities(Paithankar, Saini, Dwivedi, Sharma, & Chowdhuri, 2021). The metal causes conformational modifications in the biological molecules in the "direct" disruption. However, the heavy metal additionally culminates in "indirect" damaging because it produces reactive nitrogen as well as oxygen species which include indigenous oxidizing agents such as nitric oxide, along with hydrogen peroxide, hydroxyl and radicals caused by superoxide, and others. It has been demonstrated that heavy metals trigger signaling cascades(Engwa, Ferdinand, Nwalo, & Unachukwu, 2019).

In response to heavy metal contamination, free radicals are produced, which can lead to peroxidation of lipids, deterioration of DNA, and changes in sulfhydryl homeostasis. Additionally, modifications have been observed in metal-mediated calcium homeostasis (C. Zhang et al., 2022) as a consequence of the membrane's disruption, which activates a number of calcium-dependent processes, particularly endonucleases. The majority of investigations on the development of free radicals focuses on metallic elements such as chromium, nickel, cadmium, iron and copper(Sahoo & Sharma, 2023). The Fenton reaction that occurs between superoxide along with the hydroxyl radical is accompanied by copper, iron, vanadium, chromium, and cobalt. Fenton chemical reactions are mostly associated with peroxisomes, microsomes, as well as mitochondria(Fashola, Anagun, & Babalola, 2023).

Free radicals produced by metals induce mutagenic changes in DNA bases, demonstrating the connection between oxidative stress and carcinoma. The produced free radicals alter DNA bases(Renu et al., 2021) in a number of ways, the majority of which are pro-

mutagenic. This phenomenon demonstrates the crucial connection between the oxidative damage that metals induce and their cancerous potential. Cadmium, nickel, and arsenic have been reported to impede the processes involved in repairing damaged DNA(Goncharuk & Zagoskina, 2023). The following are examples of oxidative effects in DNA: (i) base alteration, which is determined by chromium and nickel; (ii) crosslinking, that is recognized by copper, nickel, iron, and oxidant; (iii) strand disintegration, which is determined by nickel, cadmium, chromium, and oxidant; and (iv) depurination, which is identified by copper, chromium, and nickel(Yan et al., 2021).

An assortment of antioxidant substances, both enzymatic and nonenzymatic, provide resistance against free radical assaults facilitated by metals(M Danouche, El Ghachtouli, El Baouchi, & El Arroussi, 2020). Antioxidant substances frequently mitigate iron contamination by: (i) inhibiting the molecular oxygen and/or peroxides chemical processes and chelating the ferrous ions; (ii) chelating iron and maintaining its redox state, which prevents the iron from diminishing molecular oxygen; and (iii) capturing generated radicals. One of the strongest categories of chemicals is thiols, especially glutathione, which protects cells by reducing peroxide, retaining radicals, and sustaining the redox condition of the cell(Fu & Xi, 2020). Under the condition that the daily dosage of vitamin E does not surpass 400 IU, which could be fatal, this non-enzymatic antioxidant is capable of preventing harmful effects that metals like iron, copper, and cadmium can bring to animals and in vitro settings(Ungurianu, Zanfirescu, Nițulescu, & Margină, 2021). The enhanced production of free radicals and other reactive species is a ubiquitous feature that can be exploited to identify both metal-induced cytotoxicity and carcinogenicity.

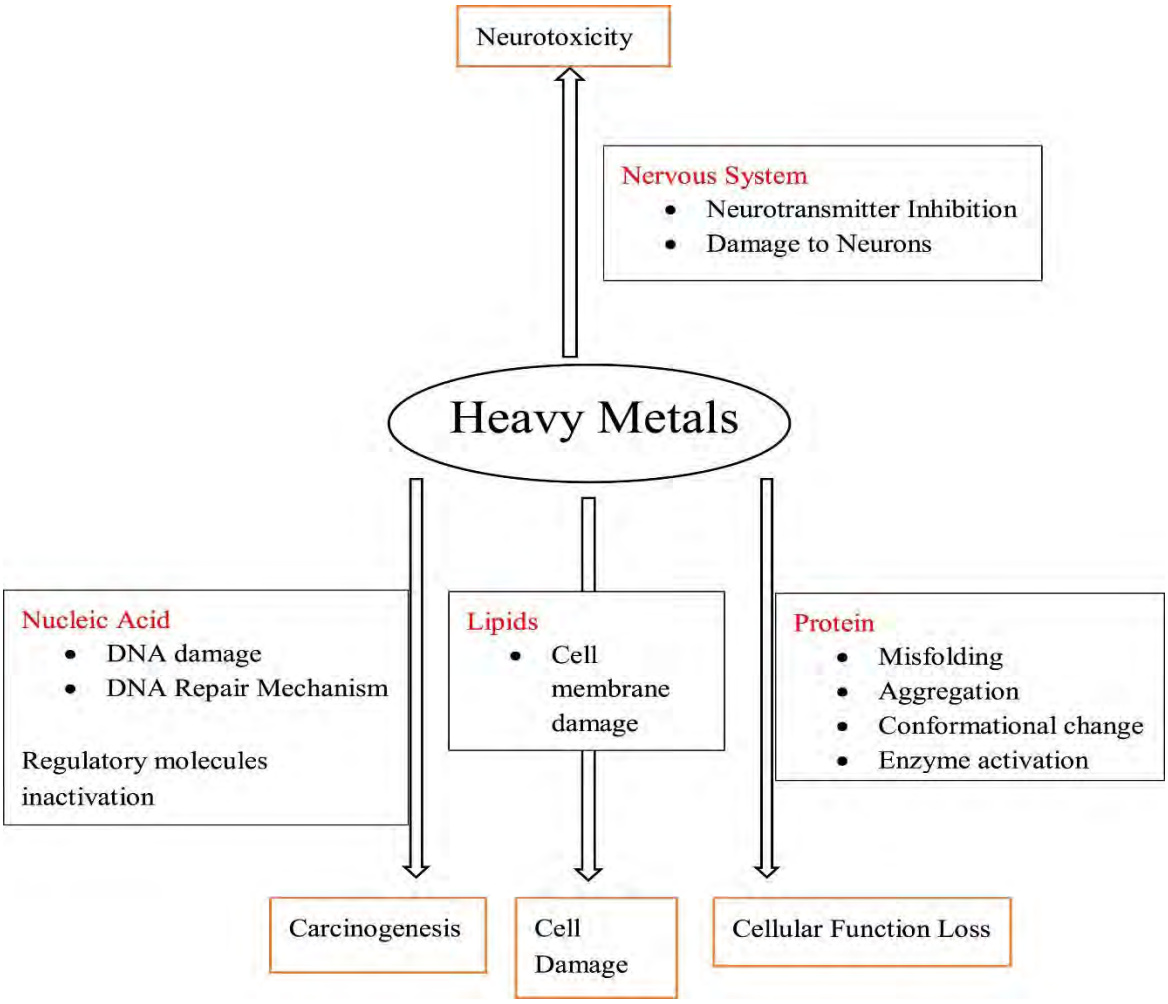


Figure 2.2 Human exposure and the biological mechanism of metallic substances(Engwa et al., 2019)

2.5 Destiny of heavy metals into the biosphere

The localization of substantial levels of heavy metals leads to an intensification in their pathogenicity. In certain places, chimneys have been elevated in order to disseminate heavy metal emissions to ensure that they don't fall into a single localized region. However occasionally it continues to have additional impacts, this increases the probability of acid rain because of its increased emissions(Zeng et al., 2023). Although the Planet Earth is perceived as a single compartment, it is actually divided into numerous additional sections, such as tiny cells or organisms. Hazardous contaminations on organisms have the ability to segregate into impenetrable deposits, which stops them from interfering with vital

metabolic processes that take place in the cell's cytoplasm. It is impossible to break away metallic substances since they are not biodegradable and subsequently endure in the ecosyplumule for an extended period of time(Khalef, Hassan, & Saleh, 2022).

When contaminants such as heavy metals accumulate in soil and sediments, they stay there for a long time before eluting into different compartments. They may also generate or deteriorate into more hazardous forms in response to reactions with other components of the soil or sediments(S. F. Ahmed et al., 2022). One illustration of this is the way that bacteria present in water, rocks, and soil combine to generate toxic methyl mercury from inorganic mercury(Walker et al., 2012). In contaminated areas like abandoned mine sites or areas where metal-containing insecticides were historically used, anthropogenic activity has generated a massive number of heavy metals. There is little flora in these places, and only strains that can withstand metals thrive there. It is occasionally necessary to "cap" these zones, which entails covering the polluted area with fresh soil and an impervious layer. Capping will assist prevent heavy metals from being absorbed by the flora and from being carried downhill by the water and entering the groundwater(Lepp & Dickinson, 1994).

In certain regions where it was utilized, remnants of the metal that contained insecticide may still include arsenic, copper, lead, and chromium. Wastewater sludge is occasionally used by farmers and incorporated into the soil; however, this might have heavy metals, particularly if the sludge originated by industrial sites(G.-h. Liu et al., 2023). High concentrations of heavy metals, including copper, zinc, lead, cadmium, and chromium, are being discovered in the earth's soil associated with these agricultural regions. Smelting releases pollutants into the atmosphere, which accumulates on the soil and creates localized contamination. Certain regions where smelting takes place exhibit dead plants and a lack of organisms like woodlice and earthworms(S. F. Ahmed et al., 2022), which aid in the decomposition of flora. Higher concentrations of clay, organic matter, and pH bind metallic substances more effectively to the soil. Less fundamental components have been discovered in more acidic soil because they dissolve more readily and seep into the earth where radicles cannot reach, depriving plants to essential nutrients(Chibuike & Obiora, 2014).

Regarding water resources, the majority of rivers are contaminated, particularly those which flow via industrial and mining zones. They subsequently flow towards the ocean, where the movement of the tide slows until they largely sink to the bottom(B. R. Singh & Steinnes, 2020). The pH level of the water has a major impact on the metals' solubility. Heavy metal-containing streams quickly enter the seawater, causing acidity levels to increase and the metals' absorption to reduce and precipitated downhill toward the bottom of the ocean(Shah, 2021).

2.6 Classification and types of heavy metals

The heavy metals are classified according to their different physical and chemical properties(Briffa et al., 2020). It is consequently commendable to categorize them based on their traits and investigate their respective categories.

2.6.1 Classification based on carcinogenicity

On the basis of carcinogenicity, heavy metals are classified into different groups. They fall into four categories according to the International Agency for Research on Cancer (IARC)(Briffa et al., 2020). which are discussed in the following.

- **Group 1**

Group 1 heavy metals have been shown to be more carcinogenic, and there is a greater correlation between their carcinogenicity and human health. The following heavy metals are categorized under this category:

- the manufacturing of aluminum,
- Arsenic and inorganic substances
- Nickel compounds,
- Nickel refining,
- Chromium VI compounds, and
- Cadmium and Cadmium compounds(Kim, Kim, & Seo, 2015)

- **Group 2A**

Possibly carcinogenic human evidence is scant, while animal evidence is abundant in group 2A heavy metals. The metallic compounds of this group includes , inorganic substances containing lead(Witkowska et al., 2021).

- **Group 2B**

This group contains Potentially carcinogenic heavy metals which have inadequate evidence in humans as well as insufficient data in animals. Group 2B heavy metals(Wallace & Djordjevic, 2020) are following:

- Vanadium pentoxide
- Molybdenum trioxide
- Methylmercury
- Metallic Nickel and alloys
- Lead
- Cobalt

- **Group 3**

Carcinogenicity of group 3 metals is not categorizable. Inadequate proof in humans, and inadequate
justification in animals' carcinogenicity levels. The heavy metals here are including,

- Chromium III compounds
- Chromium metallic compounds
- Copper
- Mercury and inorganic mercury compounds
- Selenium and selenium compounds

- Arsenic organic compounds , arsenic compounds not metabolized by humans(Mondal et al., 2017).

➢ **Group 4**

Group 4 heavy metal's carcinogenicity level is almost negligible; evidence points to neither human nor animal carcinogenic qualities(Nwokocha, Owu, Nwokocha, Ufearo, & Iwuala, 2012). Examples include.

- Manganese
- Silver
- Zinc

2.6.2 Classification of heavy metals based on varying nature

Depending On of different kinds of nature(FANI, 2023); heavy metals are classified into 5 major categories which are following.

➢ **Macronutrient heavy metals**

These heavy metals are required in higher amounts for the sustainability of a living body. These include; Iron and Cobalt(Aigberua & Izah, 2019; Siedlecka, 1995).

➢ **Micronutrient heavy metals**

They require in micro level for the body Including; Copper, Molybdenum, Nickle, Iron, Chromium, Manganese(Orman, Ok, & Kaplan, 2014).

➢ **Potentially hazardous heavy metals**

Lead, mercury, cadmium, platinum, selenium, tin, zinc, palladium, bismuth, and gold are those heavy metals which are highly toxic to almost all life forms including humans as well(Carolin, Kumar, Saravanan, Joshiba, & Naushad, 2017) .

- **Significant heavy metals**

Significant heavy metals are considered as precious metallic substances which are most expensive and used by mankind for different purposes they are Palladium, Ruthenium, Platinum, Silver, and Gold(Tunali, Tunali, & Yenigun, 2021).

- **Radioactive metallic nuclides**

Radioactive heavy metals include ; Praseodymium, Cerium, Thorium, Radium, and Uranium(Alseroury et al., 2018).

2.7 Heavy metal chromium

The earth's crust possesses chromium (Cr), a metal which exists naturally. Its oxidation levels, also known as valence states, fluctuate between chromium (II) to chromium (VI)(Jacobs & Avakian, 2005). The trivalent variant of chromium substances, [Cr (III)], are robust and can be encountered in ores like ferro chromite. Following that closest in stability is the hexavalent [Cr(VI)] compound(Patlolla, Barnes, Yedjou, Velma, & Tchounwou, 2009). Naturally occurring forms of fundamental chromium [Cr (0)] are rare. There are many different human and naturally occurring sources of chromium that permeate into the soil, water, and atmosphere, but manufacturing factories emit the greatest amount of the metal(Chrysochoou, Theologou, Bompoti, Dermatas, & Panagiotakis, 2016). The manufacturing of the chromate, tanning plant facilities, stainless steel welding procedure, ferrochrome, and chrome-plated pigments, as well as metallic manufacturing, are the sectors that contribute substantially to the chromium emissions. Elevated chromium contents in the atmosphere have been associated with chromium emissions into the environment and effluent, mostly from the chemical-based refractory, and metallurgical sectors(Ahmad et al., 2021). The predominant type of chromium that is emitted into the natural environment as a result of human operations is hexavalent [Cr(VI)](ATSDR, 2012).

Table 2.1 The characteristics and uses of Chromium(Shadreck & Mugadza, 2013)

Properties	Uses	consequences for individuals	
Density: of 7.15 g/cm3	metallic alloys	When chromium (VI) is consumed orally, it typically results in sudden intoxication and a range of symptoms, such as:	gastrointestinal ulceration,nausea and vomiting, fever, diarrhoea,
the 21st most prevalent metal on the planet's surface	metal-glazed ceramics		vertigo, toxic nephritis, liver damage,coma, death (usually at 1–3g)
recovered as Siberian red lead, which is a chromite mineral.	plating with electrodes	Persistent infection can result from prolonged skin contact or inhaling chromium (VI). chromium (VI) may result in:	allergic contact dermatitis and eczema, gingivitis, irritation of mucous membranes, bronchitis, liver and kidney disease,
robust	Leather tanning process		
lustrous, steel-grey	production of artificial gemstones		
moderately reactive metal	dye-based paints		sinusitis, pneumonia, lung cancer chrome holes, especially in the forearms, hands, fingers and nose.
reacts with the majority of acids	Glass is colored green employing chromium salts.		
creates a coating of chromium (III) oxide, which lessens the metal's corrosiveness.			

2.7.1 Types / oxidation states of Chromium

Cr exhibits multiple potential oxidation levels in complexes (which are typically spectacularly colored), including +2, +3, +4 (chromium oxide CrO_2 is known to occur very infrequently), and +6 oxidation state(Ukhurebor et al., 2021). The major forms of chromium are discussed below.

2.7.1.1 Divalent chromium

The existence of chromium in the form of +2 oxidation state is called divalent chromium. Substances with notable base characteristics are chromium (II) ox-ide and hydroxide (CrO and $Cr(OH)_2$)(Ji et al., 2023). Chromium (II) oxide appears black, and hydroxide is yellow, depending on the component. Blue hues are found in chromium (II) salts. These are typically produced from chromium (III) by redox processes. Cr^{3+} is reduced to Cr^{2+} oxidation state by the hydrogen emitted during the process. Cr^{2+} complexes have an exceptionally significant reducing potential; in certain scenarios, they can even substitute water's hydrogen as well(Qian, Li, Sun, Xaikoua, & Sun, 2020).

2.7.1.2 Trivalent chromium

Trivalent chromium is the type of chromium that exists in the +3-oxidation state. In substances, +3 is the significantly persistent oxidation configuration of chromium(Jiménez, Doistau, Poncet, & Piguet, 2021). Chromium exhibits amorphous attributes, meaning that depending on the situation, this substance may serve as either an acid or an alkaline substance. In liquid, it fails to disintegrate. Due to its relative inertness, chromium (III) oxide reacts only when heated (or smelted)(Monga, Fulke, & Dasgupta, 2022).

2.7.1.3 Hexavalent chromium

Hexavalent chromium substances, which are potent oxidizing agents, are referred to as those containing chromium that exhibit an oxidation status of +6. In this instance(Abdulmalik et al., 2023), two acids have been investigated as hydroxides: dichrome $H_2Cr_2O_7$ and chrome $HCrO_4$. They are not employed in practice and can only be found in solutions forms(HUVINEN). Consequently, their corresponding salts—

chromates and dichromates—have significant practical implications. In an acidic environment, dichromates orange in color remains resilient, while chromates (yellow) do so in an alkalinity habitat(A. Gautam, Kushwaha, & Rani, 2021).

Multiple regulatory and non-regulatory organizations have designated the hazardous commercial contaminant i.e. hexavalent chromium [Cr(VI)] as a potential human carcinogen. Proximity to chromium can have varying consequences for health depending on its oxidation level(Pooja Sharma, Singh, Parakh, & Tong, 2022). The metallic kind of chromium has moderate toxicity, whereas the hexavalent form has severe toxic effects. It was formerly believed that all substances containing Cr(VI) were artificial, while Cr(III) was found naturally in soil, water, air, and biological components(Genchi, Lauria, Catalano, Carocci, & Sinicropi, 2021). nevertheless, naturally existing Cr(VI) has recently been discovered in surface and groundwater at concentrations higher than the 50 µg of Cr(VI) per liter recommended by the WHO for drinking water(Felix, Gable, Vitale, Gratson, & Carriker, 2020). Because of its widespread utilization in so many commercial operations, chromium contaminates a wide range of ecological networks. Commercially available applications for chromium-based substances include wooden maintenance, leather tanning plants dyes and pigments, machine the welding process, and chrome plating of metals. Boilers and kitchen appliances also employ chromium as an anticorrosive(Ashour & Tony, 2020).

2.7.2 Impact of hexavalent chromium (Cr (VI)) on human health

[Cr (VI)] is a worldwide environmental contaminant that raises the incidence of several malignancies and is becoming more widely acknowledged as a neurotoxin(Wise Jr, Young, Cai, & Cai, 2022). Chromium (VI) and its metabolites—especially chromates—enter the human system through several pathways. The exposure to hexavalent Chromium can occur predominantly by consumption, respiration, and direct contact with the skin. Being subjected to Cr (VI) can be categorized into three categories: acute (lasting 14 days), transitional (lasting 75–364 days), or persistent (lasting 365 days) based upon the period that it persists(W. Yang, Song, Li, & Zhang, 2020). There are several ways that Cr (VI) could potentially prove detrimental. It can inhibit vital enzymes like oxidative

phosphorylation, interfere with enzyme function cofactor assimilation sites, diminish immune response effectiveness, and alter cellular architecture, particularly with regard to the lipoprotein component of the membranes.

2.7.3 Consequences of the hexavalent chromium compounds on plant health

Cr (VI) intoxication manifests in plants as aborted sprouting of seeds, injured radicle system, decreased radicle development, diminished biomass, shortened plant height, compromised photosynthetic capacity, membrane damage, chlorosis and necrotic to the leaves, low grain yield, and subsequently the demise of the plant(Stambulska, Bayliak, & Lushchak, 2018). The two most frequently encountered chrome oxides found in soil are CrO_{42} and $HCrO_4$, which are readily incorporated in plants and pollute soil(W. Yang et al., 2020). Cr (VI) absorption affects biomass and plant plumule lengths. While few crops remain unaffected by low levels of Cr (3.8104 M), the majority of plants are severely affected by chromium compounds, which hinder their development and yield(Shanker, Cervantes, Loza-Tavera, & Avudainayagam, 2005).

Development of plants is hindered when Cr (VI) is present because it reduces food uptake and photosynthesis. Reactive oxygen species are produced as a result of significant disruptions to a number of physical, structural, and biological mechanisms within plant cells. Two signs of Cr pollution include plant apoptosis and chlorosis(Jobby, Jha, Yadav, & Desai, 2018). The discharge of magnesium ions from the chlorophyll molecule, distortion of the ultrastructure of chloroplast , inhibition of photosynthetic electron transport chain , and suppression of the synthesis of chlorophyll are all observed(Pooja Sharma, Tripathi, Vadakedath, & Chandra, 2021). Cr (VI) poisoning in plants manifests as impaired growth of plants, apoptosis and distortion of the leaves, deterioration of the radicle tissue, chlorosis, reduced enzyme function, nutrient absorption, transportation, photosynthesis, peroxidation of lipids, shattering of DNA strands, and chromosomal distortion(Guo, Xiao, Zhou, & Chi, 2021).

2.7.4 Impact of hexavalent chromium (Cr (VI)) on microorganisms

Microbial populations and their variety are adversely affected by chromium in multiple manners. Excessive Cr (VI) contents can lower the number of microbial species by impeding the formation of extracellular polymeric substances (EPSs), electron competing, and other mechanisms. additional consequences such as an excess of reactive oxygen species (ROS), malfunctioning proteins and enzymes, thiol and iron-sulfide complex devastation, suppression of functional genes, nutrient absorption and metabolic processes, phospholipid peroxidation, damaged DNA, etc.(Bhakta, 2017).

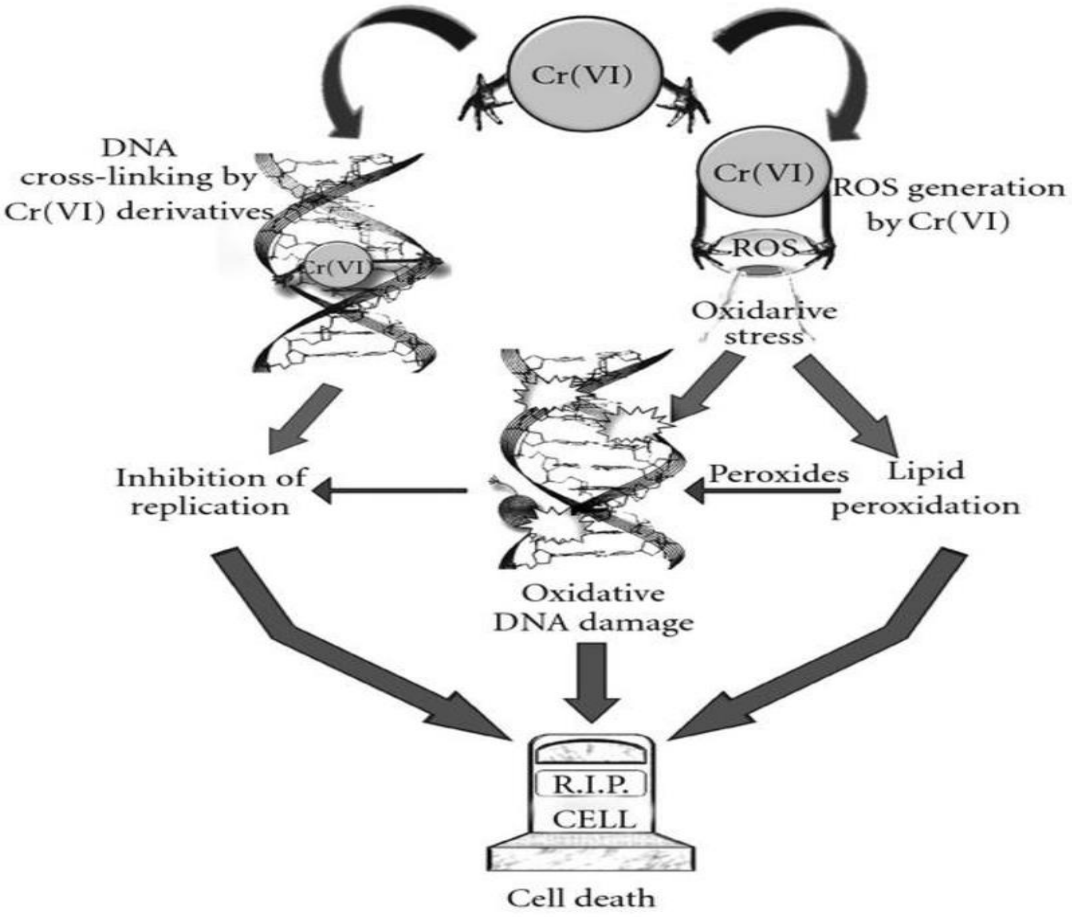


Figure 2.3 chromium induced cytotoxicity(Guha, Rajkumar, Kumar, & Mathew, 2011).

2.7.5 Microbiological treatment

Many plants and microbes have evolved a variety of defense mechanisms against the harmful effects of Cr (VI)(Z. Rahman & Thomas, 2021). The most well-understood process for such bioremediation among the different techniques is the enzymatic transformation of Cr (VI) by microorganisms into Cr (III)(S. Singh, Kang, Mulchandani, & Chen, 2008). The biological conversion of Cr (VI) to less mobile Cr (III) is mediated by or caused by chromium-resistant microorganisms and precipitation of these bacteria may be a helpful remediation technique for Cr (VI)-polluted sites. The microbial cell wall's different functional groups are bound extracellularly by the Cr (VI) ion and removed by interfacial precipitation, exchange of ions, or other comparable mechanisms(Jobby et al., 2018). By utilizing metal ions as a source of energy and transforming them into biomass, bacteria are able to effectively eliminate these contaminants from the natural world via enzyme-catalyzed toxic biochemical deterioration(GracePavithra, Jaikumar, Kumar, & SundarRajan, 2019).

Biological remediation techniques like bioaccumulation, the bioremoval process biosorption, and bioleaching processes have been reported to be effective in detoxifying chromium as well as other metals of concern from industrial waste water(Fernández, Viñarta, Bernal, Cruz, & Figueroa, 2018). In the metabolism-dependent mechanism of bioaccumulation, the hexavalent version of chromium (Cr (VI)) is only transmitted across the cellular barrier by living biomass. There are distinct steps to the bioaccumulation process in bacteria. In the beginning, potential lethal metallic ions hook themselves to the ligands on the cell's periphery. After developing on the cell surface, the metal-ligand complex is eventually carried inside via transporter enzyme(Ramli, Othman, Kurniawan, Abdullah, & Hasan, 2023).

Furthermore, metal binding proteins like metallothionein and phytochelatins collaborate with intracellularly delivered aggregates resulting in methylation, precipitation, and other processes(Yu, Lin, & Zhang, 2019). The method halts the growth of microbial cells and only functions on living cells at the more elevated metallic concentration. Additionally, industrial effluent's hazardous chromium ions are reduced and eliminated in a sustainable

manner by the mechanisms of biosorption, biological transformation, and bioaccumulation(A. Singh, Porwal, & Varma, 2021).

2.8 Heavy metal Arsenic

Arsenic is a ubiquitous element that is detected at low concentrations in virtually all environmental matrices. Anthropogenic activity and natural events like soil deterioration and erupting volcanoes both contribute to arsenic pollution in the surroundings(Dodson et al., 2018). Numerous commercially manufactured substances possessing arsenic have been utilized to create agriculturally useful products like herbicides, fungicides, algicides, insect repellents, sheep dips, hardwood preservatives, and dyes and pigments. Additionally, they have been utilized in animal healthcare to eliminate tapeworms from sheep as well as cattle. Throughout for almost a century, healthcare professionals have also employed arsenic-based substances to treat syphilis, yaws, amoebic dysentery, and trypanosomiasis(Srivastava, 2020).

Recently, Acute promyelocytic malignancy can now be treated with arsenic trioxide according to a new FDA approval as an anticancer therapeutic. Its capability to induce tumor cells to undergo the process of programmed death, or apoptosis, has been associated to its therapeutic properties(Gurnari et al., 2020). Specific tropical illnesses like African sleeping sickness and amoeba dysentery, as well as parasitic infections like filariasis in dogs and black head in poultry and turkeys, are still treated using medications based on arsenic(Sisodia, 2023). The two predominant inorganic varieties of arsenic are pentavalent arsenate along with trivalent Arsenite. Trimethyl arsine oxide (TMAO), dimethylarsinic acid (DMA), and monomethylarsonic acid (MMA) are the methylated by products that exist in organic configurations(W. Liu et al., 2017).

Table 2.2: The characteristics and uses of Arsenic (Haynes, 2014)

Characteristics	Uses	Effects on humans
Density: 5.75 g/cm3 ,	Wood preservation	lung inflammation,
5th most prevalent metal	fabrication of certain varieties of glass,	Inflammation of the gastrointestinal tract
three allotropic categories are existed.	semiconductor doping agents,	reduction in the synthesis of Wbcs and Rbcs
Vibrant silvery-gray hue	Pesticide formulations	Dermal alterations,
Highly vulnerable	explosives	elevated risk of cancer
The following minerals discovered: Arsenopyrite, Realgar, Enargite	Manufacturing of bronze	Abortions and infertility
		Cardiovascular disorders, Brain injuries, Nerve injury,

2.8.1 Oxidation states / classification of Arsenic

Among the elements in the 15 group of the 4 period in the periodic table is arsenic. It's a delicate, greenish-gray semi-metal. It can occasionally be found in the environment in its original form. The most prevalent form of arsenic metal is arsenopyrite FeAsS. Arsenic exhibits three oxidation states in substances: i.e. -3, +3, and +5(Binkowski, 2019).

2.8.1.1 Arsenic with the state of oxidation at -3

There are specific arsenic substances called arsenides that resemble salt. bearing an oxidation state of -3. These are crystalline materials that exhibit a grayish or silvery appearance along with a metallic shine. Arsenides constitute semi-conducting as well as conducting elements in massive amounts. Natural compounds can be smelted in order to generate arsenides(Maciag, Brenan, & Keltie, 2018).

2.8.1.2 Arsenic with an oxidized state of +3

Arsenic compounds possessing oxidation state of +3 are referred to as As (III). The base substance used to generate meta-arsenic acid is arsenic (III) oxide. It can be generated by smelting either a pure semi-metal or arsenic sulfide. Water may be employed to attenuate

arsenic oxides or halogenides to generate an acid i.e. or-tho-ar-senic acid H_3AsO_3, containing arsenic with an oxidation state of +3(Thomas et al., 2007).

2.8.1.3 Arsenic with an oxidized state of +5

Arsenic substances possessing oxidation state of +5 are referred to as As(V)which is a potent oxidizer. It can be produced by smelting arsenic in either elevated oxygen or in the ozone. Arsenic (V) oxide is capable of being transformed to arsenic (III) oxide via heating. The salts of arsenous acidic solution, such as sodium arse-nate (Na_3AsO_4), are frequently obtained from materials in which arsenic has an oxidation state of +5(W. Liu et al., 2017).

2.8.2 Mechanisms of potential Carcinogenicity and cytotoxicity

Evaluating arsenic's detrimental impacts is difficult since a variety of both intrinsic and extrinsic variables, like the metal's oxidation status and lubricity, greatly affect how hazardous it is. Numerous research investigations have demonstrated that the lethality of arsenic is contingent upon various elements such as species of organisms, gender, age, chromosomal vulnerabilities, exposed frequency range, time span, and individual susceptibility(Prakash & Verma, 2021). The majority of arsenic intoxication incidents in humans, plants, microorganisms, as well as marine animals, have been attributed to inorganic arsenic consumption. Compared to pentavalent arsenic (AsV), inorganic trivalent Arsenite (As III) is 2-10 times more dangerous. Approximately 200 different enzymes can be rendered inactive by As (III) by linking to thiol or sulfhydryl groups of polypeptides(N. J. Raju, 2022).

Arsenic inhibits a number of mitochondrial enzymes, which impairs respiration within cells. It also dissociates the process of oxidative phosphorylation, which is one of the techniques through which arsenic causes cytotoxicity. In in vitro conditions, arsenic interacts with sulfhydryl groups that are found in protein molecules to deactivate enzymes like thiolase and dihydrolipoyl dehydrogenase, which prevents pyruvate from being oxidized and fatty acid beta-oxidation from developing(Machado-Neves & Souza, 2023). According to epidemiological studies, prolonged exposure to arsenic accelerates the

development of carcinoma. A number of theories have been put forward to explain the process by which arsenic causes cancer. Due to its ability to elicit DNA hypomethylation, which subsequently promotes inappropriate gene regulation, arsenic may have carcinogenic effects(Ozturk et al., 2022). Furthermore, it was discovered that arsenic effectively induces the expression of the c-fos and c-jun genes and is a strong activator of exogenous signal-regulated kinase enzymes Erk1 and AP-1 transactivational activities(Srushti et al., 2023).

2.8.3 Consequences of Poisoning with Arsenic on Microbiological Behavior and Agricultural crops

Due to its association with other substances prevalent in soil, Arsenic can be detected there in a variety of forms. Consequently, a standardized indicator for assessing the soil microflora and their enzyme activity cannot be obtained from the total percentages of arsenic in the soil(Nurzhan, Tian, Nuralykyzy, & He, 2022). Microorganisms' functional groups interact to Arsenic on the cellular walls and membranes, which in turn binds to protein molecules, PO^{3-} and HO^{-} groups of nucleic acid molecules, including DNA and RNA(V. K. Sharma, Shah, Parmar, & Kumar, 2020). It culminates in a disruption of functionality and denatures the protein's structure, which hinders cell division, a particularly essential process of microbial development.

Many crops get damaged by arsenic; even trace amounts of arsenic have a variety of compromising repercussions for plants(Natasha et al., 2021). Additional ways that As may adversely affect plants include constricted radicle system, withering leaves, decreased amounts of photosynthetic pigmentation, discoloration of the leaves, and decreased levels of chlorophyll (Chl), which can alter metabolism in plants(Abbas et al., 2018). In crops planted in natural soil, concentrations of arsenic are usually low (3.6 mg kg−1)(Bora, Bunea, Chira, & Bunea, 2020). Certain crops' ability to expand and flourish is hampered by arsenic-induced phytotoxicity, which interferes with a variety of metabolic functions. Nonetheless, there are still several situations in which the terrestrial flora might acquire the As via atmospheric precipitation or incorporation via radicles from soil(Gregory, 2022).

Because of its resemblance to PO^{3-}, arsenate is one of the more prevalent constituents in the soil and is therefore vying for the transporters' attention in the radicle plasma-lemma. The most noteworthy of the various lethal manifestations examined in the current investigation was the suppression of seed germination(Allevato, Stazi, Marabottini, & D'Annibale, 2019). The high mobility of paddy rice in flood-prone areas makes it more vulnerable to As accumulation compared to any other crop(Khanna, Jamwal, Gandhi, Ohri, & Bhardwaj, 2019). A prior study has documented that arsenic intoxication results in a decline in yields of wheat owing to a drop in amylolytic efficiency(X. Liu, Zhang, Shan, & Zhu, 2005). The majority of the aquatic life that are affected by arsenic bioaccumulation are microalgae along with aquatic crustaceans(Kuehr, Kosfeld, & Schlechtriem, 2021).

2.8.4 Arsenic potential to affect human health

Globally, it is speculated that millions of individuals are habitually subjected to arsenic, especially in areas with elevated levels of arsenic pollution in the ground water, such as India, Bangladesh, Chile, Uruguay, Mexico, and Taiwan. Arsenic consumption can happen orally (by ingesting by breathing or coming into encounter with the skin, and even parenteral means(Gupta et al., 2022). Significant amounts of arsenic exposure are concerning since arsenic can have a variety of detrimental consequences on human health. Countless epidemiological investigations have revealed a robust correlation between prolonged contact with arsenic and heightened chances of carcinogenic and chronic health consequences. Almost each organ is impacted by arsenic contact, which comprises the neurological, hepatobiliary, respiratory, gastro-intestinal, dermatological, and cardiovascular system(Shaji et al., 2021). Moreover, investigations have shown that in several locations Wherever there is an arsenic emission, the usual death rates for malignancies of the gallbladder, the kidneys, the epidermis and liver are much higher. The chemical nature of arsenic influences the intensity of harmful health consequences, which are also dose- and time-dependent(Siddiqui et al., 2020).

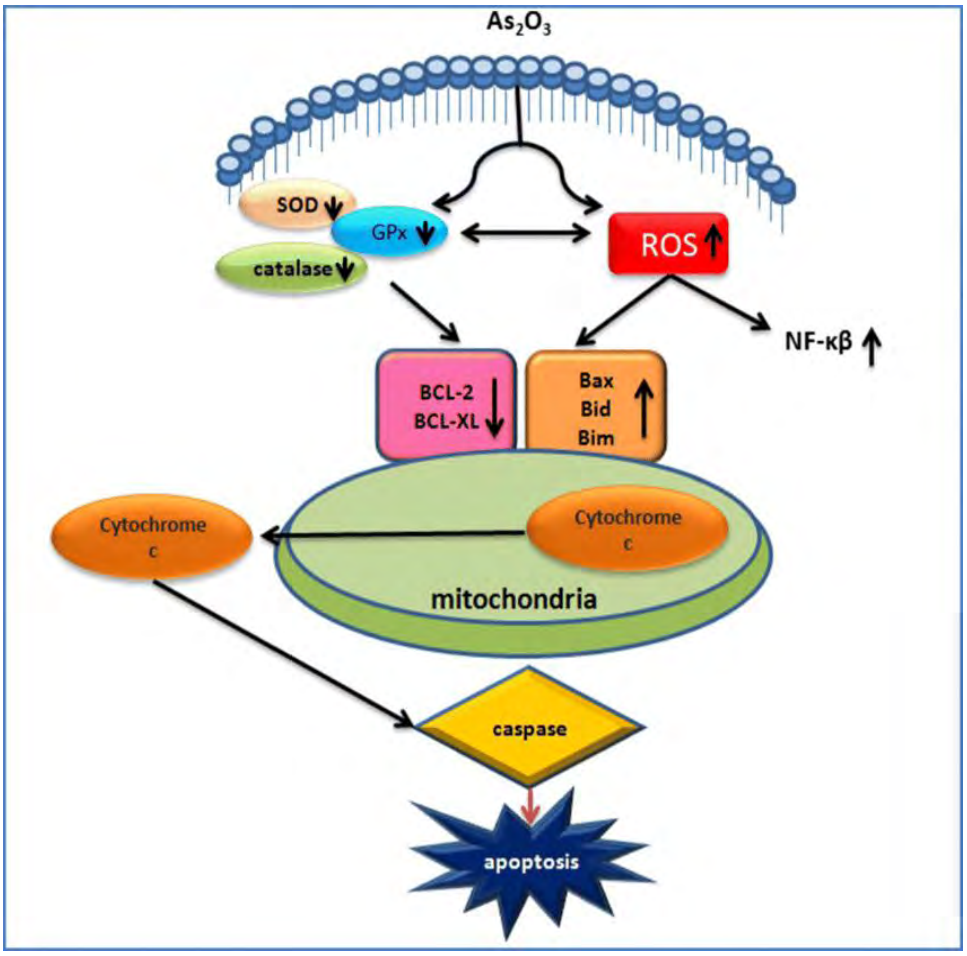


Figure 2.4: Arsenic induced cytotoxicity (Medda, Patra, Ghosh, & Maiti, 2020).

2.8.5 Extrusion / bioremoval of Arsenic by prokaryotic and eukaryotic cells

Almost all living creatures have arsenic efflux mechanisms, which have been developed to remove hazardous metalloids from cells. The genes responsible for detoxifying substances from arsenic are often transcribed by Ars operating system in bacteria and archaea. Arsenite is eliminated from the cytosol of fungus, plants, and mammals, including human beings, by multidrug resistance polypeptide (MRP) substitutes in the form of the reduced glutathione (GSH) conjugated $As(GS)_3$ (Ganie, Javaid, Hajam, & Reshi, 2024).

There are two fundamental processes for Arsenite extrusion in prokaryotes. One of these is transporter-mediated efflux through an Arsenite transporter protein, whereby energy is transmitted by the cellular membrane potential; the subsequent process is Arsenite-translocating ATPase enzymatic system. There has now been determined to be two distinct groups of Arsenite vectors(J. Zhang et al., 2022). A transmembrane protein that is resistant to Arsenite was discovered in the Epidermal portion of Bacillus subtilis and is also present in certain other species of bacteria, archaea, and fungus. Although ArsB, located throughout most Ars operons, is the mechanism that the vast majority of bacteria employ to discharge Arsenite(Bhardwaj, 2022). The co-expression of ArsA and ArsB results in the formation of an ArsAB complex, which must be present in order to be associated with ATP. Arsenite is extruded by certain bacteria with three-gene ARSRBC operons and five-gene ARS RDABC operons that use the ArsAB pump (ArsR and ArsD, which are As(III)-responsive suppressors of the Ars operons that differ)(Banerjee, Tabassum, Debnath, Hazra, & Pal, 2022).

In Eukaryotic cells components belonging to the ABC family of transporter ATPases that belong to the MRP (multidrug resilience-associated protein) category give Arsenite tolerance. which generally facilitate the transfer of GS-conjugates like leukotriene C4 (LTC4).(Steinmetz-Späh, 2023) Arsenite promoted the evacuation of glutathione from the cells that MRP1 triggered, indicating that MRP1 serves as a transporter of As(GS)3(Recio-Vega et al., 2021). MRP2 may be a key pathway associated with human arsenic decontamination as it forms bile from the liver's secretion of arsenic-glutathione aggregates(J. R. Zhou et al., 2021). It has been demonstrated that eukaryotic microorganisms possess arsenic tolerance due to MRP homologous proteins. Enhanced level of pgpA, particularly expresses an MRP homolog, is observed in Arsenite-resistant varieties that were chosen in vitro. In consequence of this, both of these mechanisms operate independently of one another and offer different routes for the recuperation of arsenic(Van den Kerkhof et al., 2021).

Material and Methods

The purpose of the current research was to determine the differential toxicological analysis of heavy metals [Cr (VI) and As (III)], on bacterial strains including the bacterial strain's detoxification of heavy metals in shake flask fermentation. The toxicity tests were also applied on plants and animal cells. All these studies are performed in The Microbial Biotechnology and Bio Engineering Lab, Department of Microbiology, Quaid-i-Azam University, Islamabad, Pakistan.

3.2 Chemicals

The chemicals used in the analysis were heavy metals, Chromium (vi) (Potassium Dichromate) obtained from DAEJUNG, and Arsenic (III) (Sodium Arsenite), which is obtained from Sigma-Aldrich for the experimental purpose. The general characteristics of these heavy metals are described in the following table 3.1.

Table 3.1 Characteristics of heavy metals used in the experiment.

Properties	Heavy metals	
Common name	Chromium (VI)	Arsenic (III)
Chemical name	Hexavalent chromium	Trivalent arsenic
Compound used	Potassium Dichromate	Sodium Arsenite
Chemical formula	$K_2Cr_2O_7$	$NaAsO_2$
Oxidation state	+6	+3
Category	Antiseptic, insecticide, herbicide	Carcinogenic
Case NO.	7778-50-9	7784-46-5
Solubility	Potassium dichromate is soluble in water and ionizes in the dissolution process.	Solubility in water is 10%
Color	Bright red orange	Grayish-white powder
Molar mass	294.18g/mol	129.91g/mol
Source	Daejung Co., Ltd.	Sigma Aldrich

3.1 Sample collection

The isolated strains of *E. coli* and *B. subtilis* were obtained from Microbiology lab, Quaid-e- Azam university, and stored at 4°C as pure culture on agar plates for bacterial analysis. similarly, the seeds of plants, *solanum lycopersicum* and *Eruca sativa,* were obtained from the vendor and eggs of *Artemia salina* were obtained from Bioinformatics lab, Quaid e Azam university, to carry out the toxicological assessment and comparative evaluation of selected heavy metals.

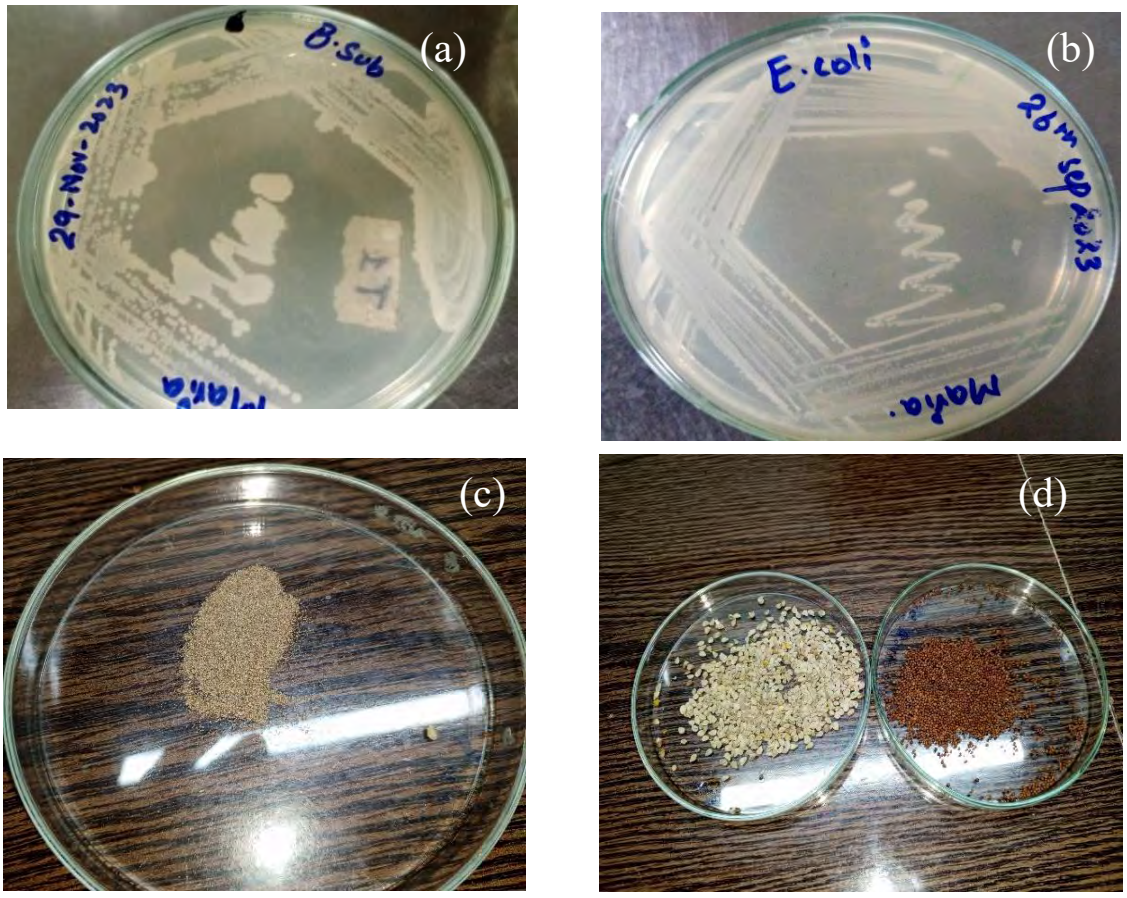


Figure 3.1 (a) fresh isolated culture of *B. subtilis*, (b) isolated culture of *E. coli,* (c) collected eggs of brine shrimp, (d) collected seeds of plants.

3.3 Toxicological implications of heavy metals on the growth of bacteria

3.3.1 Media used for the maintenance of bacterial culture and growth inhibition analysis.

In this section of the experiment, two bacteria strains i.e. *E. coli* and *B. subtilis* were used to formulate heavy metals toxicity against microorganisms. To carry out this experiment, Luria broth (L.B) medium was applied for the pre-inoculation of pure culture of bacteria as well as for the growth inhibition assay. the ph. of the medium was adjusted to 7.0. and it consists of different concentrations of chemicals for the cultivation of bacterial strains. describes in the following.

3.3.1.1 Composition of the L.B medium

- Tryptone; C_3H_5NO (10.0g/L),
- yeast extract (5.0 g/L),
- sodium chloride; NaCl (5.0 g/L)

3.3.2 Procedure

3.3.2.1 pre- inoculation

After the preparation of medium in the distilled water the colonies of bacterial culture were taken from the stored pure bacterial culture and mixed in the Luria broth medium in the flasks and then placed in the shaker incubator for 48 hours at 35 °C.

3.3.2.2 Toxicity analysis

For the toxicity analysis of heavy metals, the above pre-inoculated broth cultures of bacterial strains were employed. The experiment was applied in the Erlenmeyer flasks, in which different concentrations of heavy metals were prepared from syringe filtered stock solutions. Each flask contains the respective heavy metal concentration along with the 100 ml Luria broth medium. Every flask was inoculated with 5ml of respective bacterial strain from the 48h prepared broth culture. The composition of heavy metal concentration applied was same as discussed in the plant section. The only difference lies in the use of

broth media instead of distilled water so that the bacterial growth was facilitated properly. Then the flasks were placed for incubation in the shaker for 48 h at 35-37 °C. The samples were then withdrawn and checked for the bacterial growth by determining the optical density (A^O) at 600nm 0f wavelength by using a UV-Vis spectrophotometer. A culture- free heavy metal -containing medium was used as a blank for each concentration separately for each sample, to check the absorbance on spectrophotometer at three different time intervals i.e. 0h, 24h, 48h. Similarly, a control experiment was also applied, containing heavy metal-free bacterial culture medium for each microbial strain and checked for absorbance at 600nm. To determine the percentage of bacterial growth inhibition under varying concentrations of heavy metals, the readings were compared with the control sample. The percentage for growth inhibition was calculated using the following formula.

$$\text{Inhibition (\%)} = \frac{O.D \text{ in the control sample} - O.D \text{ in the heavy metal sample}}{0.D \text{ in the control sample}} \times 100$$

The concentrations of heavy metals used in this section of experiment are in the following table.

Table 3.2 Concentrations of heavy metals employed in bacterial toxicity assay.

1-	**1 µg/L**
2-	**10 µg/L**
3-	**500 µg/L**
4-	**1000µg/L**
5-	**50mg/L**
6-	**100mg/L**
7-	**200mg/L**
8-	**300mg/L**
9-	**500mg/L**
10-	**700mg/L**

Figure 3.2 Shake flask fermentation setup to check the toxicity of heavy metals via bacterial specimens.

3.4 Bioremoval analysis of heavy metals by different microbial strains

The selected bacterial strains were subjected to examine their degradation abilities against Chromium (VI) and Arsenic (III) heavy metals BY spectrophotometric method at different wavelengths of respective heavy metals.

3.4.1 Spectrophotometric method for chromium analysis

3.6.1.1 Preparation of reagents

- ***0.2 M H_2SO_4:*** was prepared by adding 1.064ml of 99% concentrated H_2SO_4 in 100ml of distilled water.
- ***0.125% 1,5-Diphenyl carbazide (DPC):*** 0.125% DPC solution was prepared by mixing 0.125g of DPC reagent in 100 ml of acetone.

3.4.1.2 Procedure

The analysis was performed in cleaned test tubes containing supernatant samples of each heavy metal concentration. Similar to the technique employed by (Lace, Ryan, Bowkett, & Cleary, 2019). For this purpose, the samples were withdrawn from each flask into the separate Eppendorf and centrifuged to get supernatant having heavy metal concentration and media. Then the solutions were prepared in separate test tubes. each solution contained above prepared 500µL of 0.2M H_2SO_4., 500 µL of DPC solution. And specific supernatant sample of each heavy metal concentration. By adding DPC solution the color changed will be observed immediately from colorless- pink or dark purple. Then the test tubes were gently shaken and left for 5 minutes. After the given period of time the samples were checked for absorbance or O.D at 540nm of wavelength. A heavy metal concentration- free solution consisted of above discussed reagents along with the distilled water instead of enzyme sample was used as a blank. This test was performed at regular time intervals of three days i.e. 0h, 24 h and 48h. and the results were compared as initial and final readings on UV- Vis spectrophotometer.

3.4.2 Spectrophotometric method for Arsenic (III) determination

3.4.2.1 preparation of reagents

- ***0.4 mol/L HCl:*** for the preparation of 0.4 mol HCL the concentrated solution of hydrochloric acid was diluted. By adding 354ml HCl in 1000ml distilled water to make 35.4% solution then dissolve 11.44ml in 100ml distilled water to make 0.4 molar concentration.
- ***2 % Potassium iodide:*** 2% solution of potassium iodide was prepared by dissolving 2g of potassium iodide solvent in 100 ml distilled water.
- ***2 mol/L sodium acetate:*** 2 molar sodium acetate solution was prepared by dissolving 27.216 g of reagent in 100 ml of distilled water.
- ***Azure B:*** 0.1 g of azure B reagent was added in the 100 ml of distilled water to make 0.1% of the solution.

3.4.2.2 Procedure

The protocol applied was same as used by (Cherian & Narayana, 2005) for all the concentration of selected heavy metals. The solutions were prepared in different test tubes by adding bacterial- free supernatant sample which was prepared by withdrawing from flasks (prepared for inhibition assay) and centrifuged for 10 minutes at 4 °C. and 10000 rpm. The test tubes contain 250µL specific heavy metal concentration supernatant sample, along with the reagents prepared previously i.e. the 250 µL of potassium iodide, 250 µL of 0.4mol HCl, the mixture of all theses was gently shaken and then 250 µL of azure B and 500 µL of sodium acetate solutions were added. At the last 1ml of distilled water was added. Then left for 5 minutes and measured optical density at 644nm of wavelength by using spectrophotometer. A bacterial- free and heavy metal free, containing all the above discussed reagents, solution was used as a blank. This experiment was also recorded for three regular intervals of 0h, 24h, and 48h. and then the results were compared between initial and final readings.

3.5 Assessment of bacterial membrane integrity

The effect of varying concentrations of heavy metals on the membrane integrity of selected isolated strains of *E. coli* and *B. subtilis* was assessed by measuring the lactate dehydrogenase assay.

3.5.1 Lactate dehydrogenase analysis (LDH)

The purpose of this experiment was to determine the bacteriolytic activity of heavy metals on the selected bacterial strains treated with the varying concentration of heavy metals by determining the activity of lactate dehydrogenase in bacterial cells. LDH is an enzyme which is present in the membrane of bacterial cells. Its presence determines the membrane integrity and permeability. The increase in the enzyme activity is the indication of membrane damage by the heavy metals and the enzyme will leak out of the cell into the surrounding medium.

3.5.1.1 Preparation of reagents

- ***30 mM sodium pyruvate***: 30 mM solution of sodium pyruvate was prepared by dissolving0.33015g of sodium pyruvate reagent in 100 ml of distilled water.
- ***0.2 mM NADH:*** 0.2 mM NADH was prepared by adding 0.015g of reagent in 100 ml distilled water.
- ***0.2 mM HCl:*** 612 µL of 99% concentrated HCL was added in 100 ml of distilled water to prepare 0.2 mM HCl.

3.5.1.2 Procedure

To proceed LDH analysis 100 µL of 30 mM sodium pyruvate, 2.8ml 0.2 M HCl, were added into the 100 µL of supernatant sample containing respective heavy metal concentration in different test tubes. Then finally 100 µL of 0.2 mM of NADH solution was added to complete the reaction. A heavy metal -free solution containing all the above reagents was used as a blank. Then absorbance was recorded at 340nm of wavelength using a UV-Vis spectrophotometer. The value of absorbance indicated the concentration of LDH enzyme released in the medium by rupturing the bacterial cell wall. The results of the heavy metals treated samples were then compared with the control sample to infer the bacteriolytic activity of heavy metals on selected strains by recording the activity of lactate dehydrogenase.

3.6 Growth kinetics of bacterial strains

The isolated bacterial strains were then treated with the selected concentrations of the heavy metals and analyzed for their growth kinetics. The setup of the experiment applied was same as used in the inhibition assay discussed above. The bacterial strains were treated with different concentrations of heavy metals for about 7-9 days. The growth curves were obtained in L.B medium in the presence of heavy metals i.e. Cr (VI), and As (III) by growing the cells in shake flask experiment and their optical density was determined by using a spectrophotometer at 600nm of wavelength. The readings were then measured at regular intervals of 7-9 days until the densities of the cells reached their stationary phase.

The concentrations of the heavy metals used in this section were the same as applied in the toxicity analysis of bacteria discussed in the above section.

3.7 Fourier transformed infrared spectroscopy of bioremoval of heavy metals.

Employing an Agilent Cory 630 FTIR spectrophotometer, FTIR investigation was carried out to check at the modifications to the structure of Cr (VI) and As (III) and to anticipate for the shifts in the chemistry of both heavy metals simultaneously prior and after transformation. The wavelengths of the transmittance spectrum have been determined between 4000 and 515 cm^{-1}. The treated sample's supernatant was utilized for this purpose, and 50 mg concentrations of both heavy metals were measured.

3.8 Biosorption analysis of heavy metals by bacterial specimens via FTIR

After being exposed to heavy metals, *B. subtilis* and *E. Coli* were centrifuged, pelleted, and subsequently rinsed thrice with the phosphate buffer solution. The cells in the pellets were lyophilized after being revived in PBS. The preserved bacteria were then investigated using FTIR Spectroscopy.

3.9 Toxicological assessment of heavy metals against different plants

3.9.1 Preparation of stock solutions for Phyto toxicological analysis

In this study stock solutions of 10,000mg/l were prepared for both heavy metals, i.e., Cr (VI) and As (III) from potassium dichromate ($K_2Cr_2O_7$) and Sodium Arsenite ($NaAsO_2$), respectively in 100 ml distilled water by adding 1 g of both compounds. Afterwards both stock solutions were purified by Syringe filtration method and then stored at 4 °C for experimental purposes. Then different concentrations of both heavy metals [Cr (VI) and As (III)] were prepared from 1000mg/L stock solution which was prepared from the above prepared stock solutions of 10,000mg/L in separate flasks. Following concentrations were used in the experiment.

Table 3.3 heavy metals concentrations used in the Phyto toxicological analysis.

1 µg/L
10 µg/L
500 µg/L
1000 µg/L
50mg/L
100 mg/L
200 mg/L
300 mg/L
500 mg/L
700 mg/L

3.9.2 Procedure

For this experiment different Petri plates containing watt man filter paper in each plate were prepared having a marked line on the filter paper. The plants used were *solanum lycopersicum* (tomato) and *Eruca sativa* (Taramira). The seeds of both plants were first washed with the washing agent and then with the distilled water. The washing agent used for this purpose was prepared by mixing bleach and Tween (viscous liquid) into the distilled water. After washing the seeds were placed in petri plates on filter paper prepared previously, and then soaked with 5ml solution of all the prepared stock solutions one by one. The petri plates were then placed under 60-100 watt of lamp. On daily basis, 5ml of each of the respective concentrations of heavy metals were added in the plates so that the seeds would not be dried off and took the readings of radicle and plumule length of growing seeds after seven days. A heavy metal free plate of both seeds was prepared as control sample to compare the results plants growing in the presence of heavy metals with the control sample and to calculate the percentages (%) of seed germination rate, radicle length inhibition degree (%), plumule length inhibition degree (%), and dry biomass inhibition (%) from control sample.

3.9.3 Toxicity assay

The plant's development was determined by the toxic effects of heavy metals by calculating the degree of inhibition for plant radicles and plumule length, plant's dry biomass germination degree of seed. The following formula was applied.

$$\text{IH (\%)} = \frac{X_{\circ} - X}{X_{\circ}} \times 100$$

Where X_0 is a variable (control sample) in the absence of heavy metals, containing distilled water and X is the variable in the presence of heavy metals. IH is the inhibition degree of respective plant.

3.9.4 Biosorption analysis of heavy metals to the plants by FTIR

The plants were also subjected to assess the absorption of selected heavy metals by utilizing Cory 630 Fourier transform infrared spectrophotometer. For this purpose, the plants were dried and crushed into a fine powder for both heavy metals utilizing the 50 mg concentration in each case. Afterwards the crushed plant samples were subjected to FTIR analysis in the form of solid samples.

3.10 Lethality assay for *Artemia salina* (brine shrimp)

3.10.1 Preparation of reagents.

Heavy metals concentrations. The concentrations of both heavy metals from 10,000 prepared stock solutions were prepared by the same procedure as discussed in the plant section. Moreover, some extra heavy metals concentrations were also added in this section to check the activity of artemia salina on lower concentrations as well. For this purpose, the extra micrograms concentrations were prepared from 250µg stock solution which was prepared from 1000 mg/L stock solution for both selected heavy metals. The following concentrations were used in this section of experiment.

Table 3.4 concentrations of heavy metals employed in brine shrimp lethality assay.

1 µg/L
2 µg/L
4 µg/L
6 µg/L
8 µg/L
10 µg/L
250 µg/L
500 µg/L
750 µg/L
. 1000 µg/L
. 50mg/L
. 100 mg/L
. 200 mg/L
. 300 mg/L
. 500 mg/L
. 700 mg/L

3.10.2 Procedure

The saline solution was prepared by adding 4.5g of salt (NaCl) in 500ml distilled water. For the time being the flask was placed on a magnetic stirrer to mix the solution completely and 1 g of eggs were added in it. An air pump was fixed in the flask so that the flask was facilitated with aeration at room temperature and placed under the lamp. This is because the eggs of brine shrimps need a proper aeration to hatch and release the nauplii. Then after 24h the hatching of eggs was observed by turning off the lamp and air pump. Again, it was placed for the next 48h until the hatched nauplii would completely mature.

After 48 h different test tubes, containing different concentrations of heavy metals in each, were prepared and then 10-20 matured nauplii were added in each concentration. Then after every hour the number of live and dead cells was observed to compare the toxic intensity of each heavy metal according to time.

Results

The research was conducted to confers the differential toxicological assessment of heavy metals i.e. chromium (VI) and Arsenic (III) on isolated bacterial strains of (*B. Subtilis* and *E. coli*), as well as to valuate toxicity rate of heavy metals via isolated bacterial strains. The analysis was also performed on different plant seeds i.e. (*Solanum lycopersicum and Eruca sativa*) as well as on the animal cell i.e. *Artemia salina* (brine shrimp). The experimental research was divided into certain attributes including a) elucidation of Phyto toxicological analysis of heavy metals. B) evaluation of lethality associated with the heavy metals to animal cells. C) analysis of Bacterial growth by the action of heavy metals via spectrophotometric method d) detoxification/ transformation of heavy metals by potential microbial strains. e) evaluation of bacterial membrane integrity via LDH assay, and at last used some analytical technique such as FTIR, for confirmation of result. F) demonstration of bacterial growth kinetics through effective concentration of heavy metals via spectrophotometric method G) determination of biosorption of heavy metals to the bacterial cells as well as plant cells.

Cytotoxicity of heavy metals on the growth of bacterial cell

After the pre- inoculation of bacterial pure cultures, the toxicity analysis was performed on both isolated bacterial strains to check the cytotoxicity of heavy metals on the selected bacterial strains and to confers bioremoval rate of heavy metals by microbes. The shake flask fermentation experiment was employed for this purpose. In order to determine growth, freshly cultivated pre-cultures were inoculated for 24 hours before being transferred into flasks including control (no heavy metal was added) and examined bacterial strains that had been inoculated with varying concentrations of heavy metals and the spectrophotometer was used to quantify the absorbance of growth at 600 nm, which was then graphically plotted. The growth rate was observed at three regular intervals of 0, 24, and 48hrs. The bacterial strains of *E. coli* and *B. subtilis* developed slowly at first by lengthening its growth phase, however as different concentrations were introduced, the growth duration climbed progressively and reached 48 hours.

Effect of heavy metals on the growth of *E. coli*

The growth response of *E. coli* to varying heavy metals were illustrated in the fig 4.1 In case of *E. coli* treated with the Cr (VI) heavy metal, it was observed that the growth rate at all the other selected concentrations was higher than the control (1.766) except at 200,300, 500, and 700

mg/L concentrations, which showed lower growth than the control. however, in treatment with As (III) the only µg/L concentrations showed higher growth than control while at all other mg/L concentrations, the reduced growth rate was observed. In this case the As (III) showed more toxic behavior to *E. coli* than that of Cr (VI).

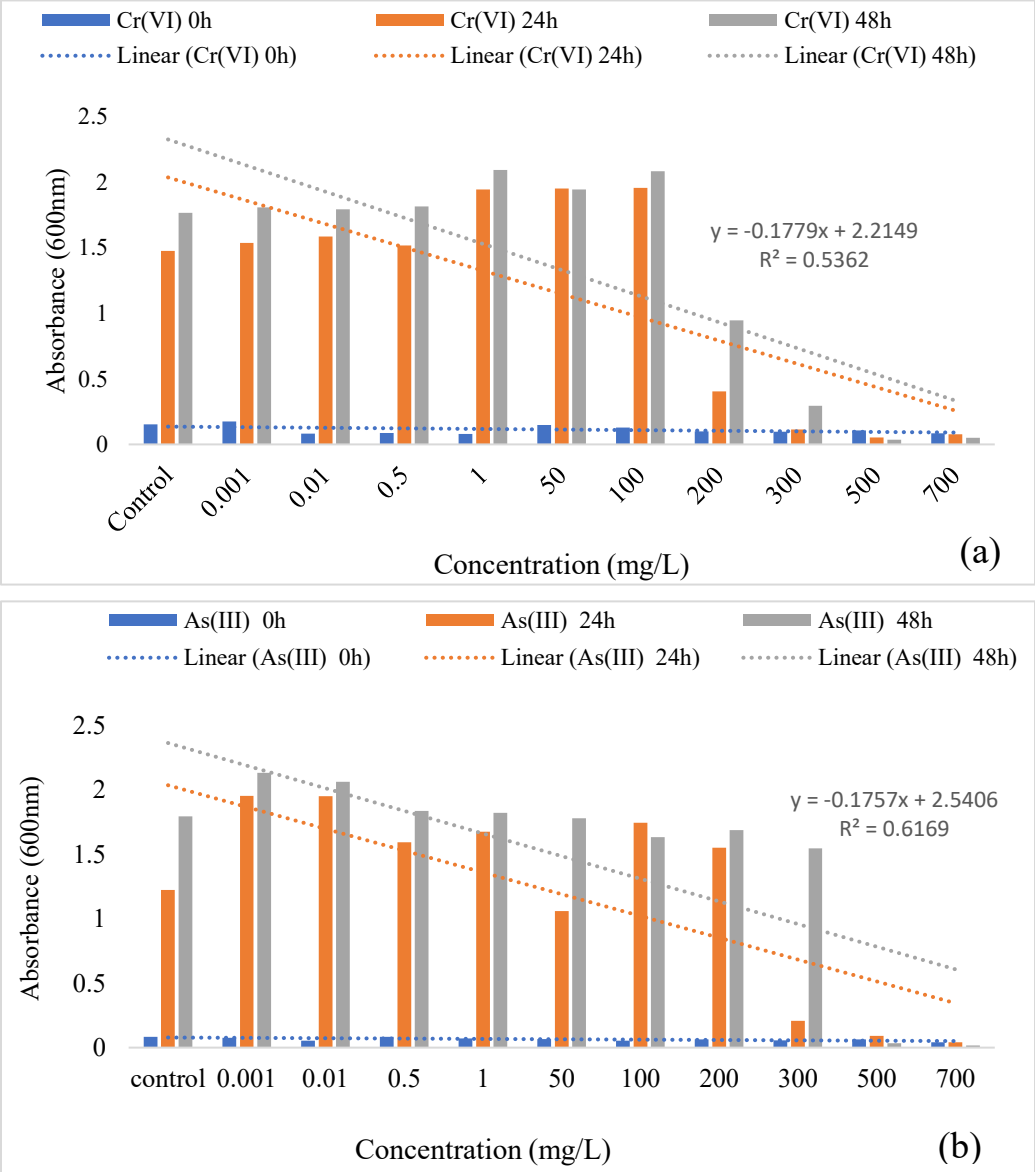


Figure: 4.1 Effect of Heavy metals on the growth of *E. coli*, (a) effect of Cr (VI), (b) effect of As (III).

Effect of heavy metals on the growth rate of *B. subtilis*

In the case of growth, the response of *B. subtilis* to varying concentrations of heavy metals showed somehow different behavior than *E. coli*. in treatment with different concentrations of Cr (VI) the *B. subtilis* showed, as it showed lower growth on all the concentrations than the control. However, in case of treatment with As (III), 1 µg/L concentration showed the growth rate of 1.92 which is greater than the control i.e. 1.786 and almost all the concentrations showed greater growth than the control except higher concentrations of 500mg and 700 mg. this behavior indicates that *B. subtilis* showed greater sensitivity in treatment with chromium than that of arsenic (III). The toxic effects of heavy metals on *B. subtilis* were shown in fig. 4.2.

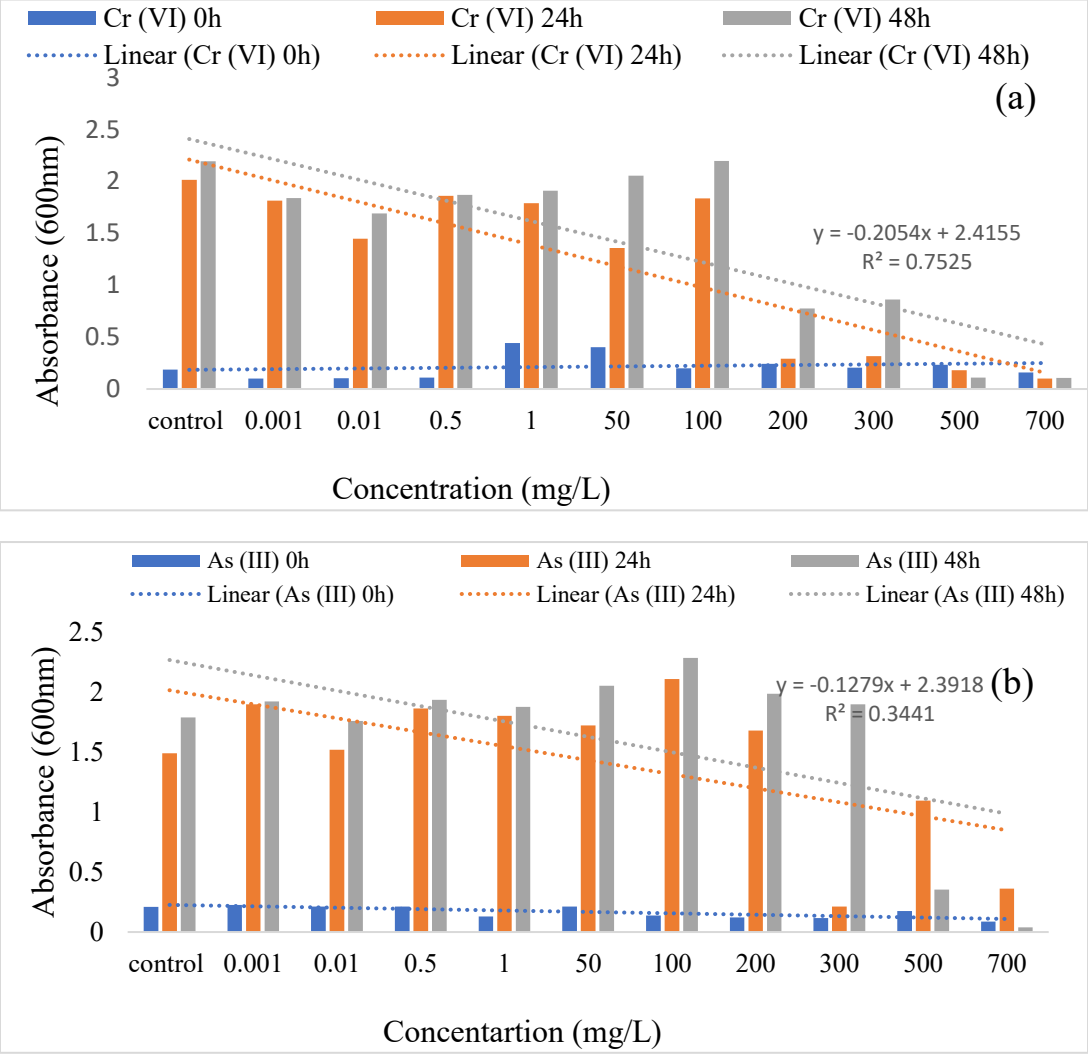


Figure 4.2 Effect of heavy metals on the growth of *B. subtilis*. (a) Effect of Cr (VI), (b) effect of As (III).

Bioremoval analysis of heavy metals by selected bacterial strains

The selected heavy metals were subjected to check their bioremoval rate via isolated bacterial strains. For this purpose, the spectrophotometric methos was used in which Specific chemical reactions were employed separately for both heavy metals i.e. Cr (VI) and As (III) which is already discussed in methodology. The absorbance of Cr (VI) was observed at 540nm and As (III) was observed at 644nm via spectrophotometer.

Bioremoval rate via *E. coli*

the ability of *E. coli* was determined for the bioremoval of both heavy metals at selected concentrations in case of Cr (VI) the higher bioremoval rate was detected at 1 µg/L, i.e. 98% via *E. coli* but this transformation rate was decreases at higher concentrations of heavy metal. However, in case of As (III) the highest transformation was detected at 10 µg/L, and the lowest was at 1 µg/L.

Bioremoval rate via *B. subtilis*

In the other case, the highest Cr (VI) transformation of 83% was detected at 1 µg/L, while the lowest of 3% transformation was noted at higher concentration of 700mg/L via *B. subtilis*. on the other hand, the bacterium *B. subtilis* displayed the highest bioremoval of 98 % at the higher concentration of 700mg /L in case of As (III) heavy metal. However, all the remaining concentrations of both heavy metals reflected random variations in the bioremoval rate of heavy metals.

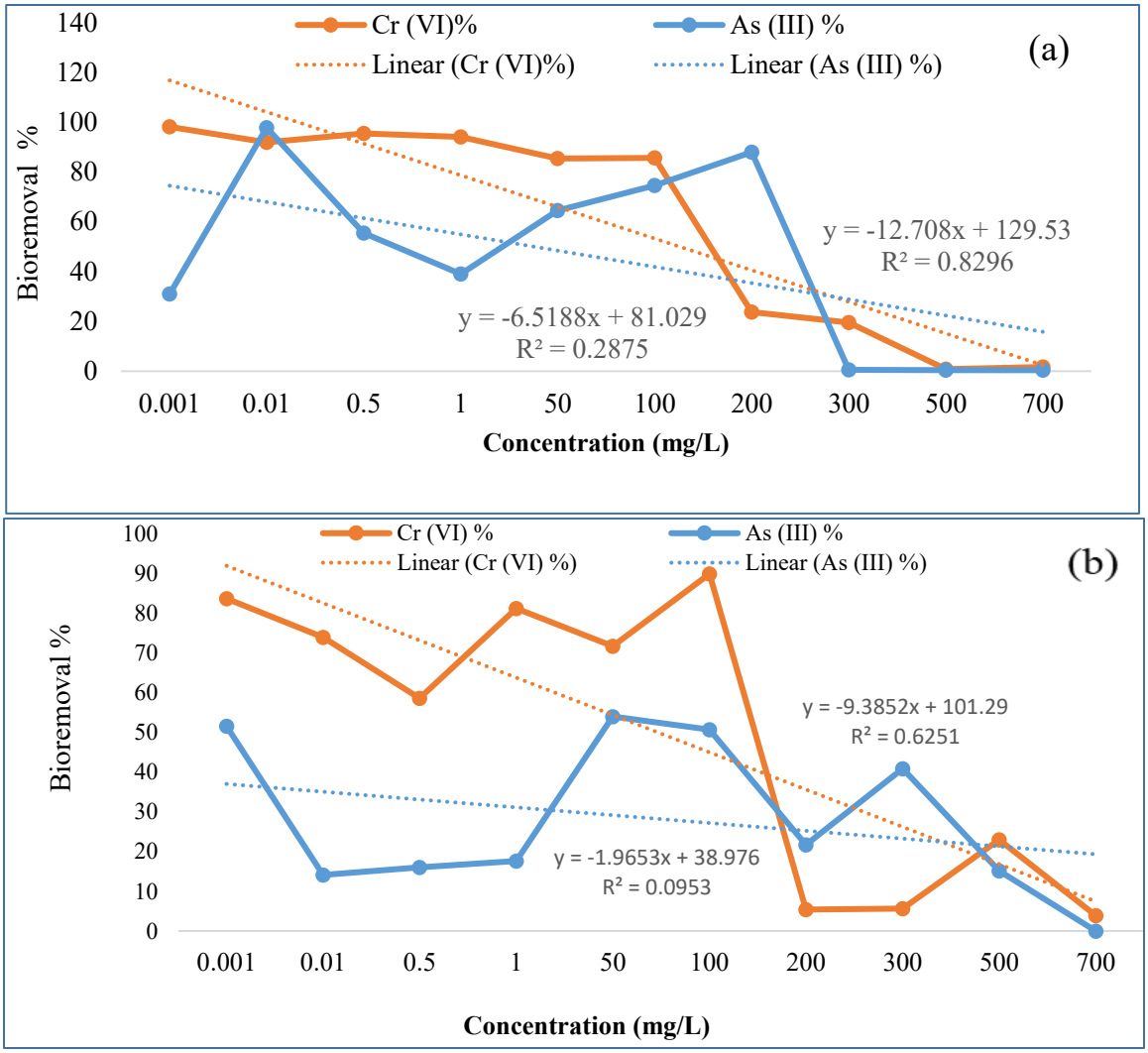


Figure: 4.3 UV-Vis spectroscopic bioremoval analysis of heavy metals (a) via *E. coli* (b) via *B. subtilis*

Effect of heavy metals on the bacterial membrane integrity

The activity of Lactate dehydrogenase was used to investigate the effects of the Cr (vi) and As (III) on the structural integrity of the membranes of *E. coli* and *B. subtilis*.

Lactate dehydrogenase analysis

The enzyme lactate dehydrogenase (LDH) analysis was used to measure the detrimental effects that heavy metals brought to microbial cells. Multiple heavy metal concentrations were examined for LDH absorbance because this enzyme is commonly employed as a stress indicator to identify tissue damage caused by xenobiotic chemicals. The membrane of the bacterial cells got damaged by all

concentrations of Cr (VI) and As (III) heavy metals, but the heavy metal Cr (VI) caused greater damage to both bacterial cells i.e. *E. coli* and as well as *B. subtilis* than that of As (III) at higher concentrations significantly, compared with the control. Fig 4.4 showed the absorbance of heavy metals on the release of LDH enzyme from the bacterial cells. In treatment with the Cr (VI), *E. coli* showed greater release of LDH enzyme i.e. (0.34195) at the concentration of 700mg/L than that of As (III) i.e. (0.014854) at the same concentration. similarly in the case of *B. subtilis* the 0.35275 absorbance was observed at 700mg/l in treatment with Cr (VI) which is greater than that of the As (III) i.e. 0.1016 at the same concentration.

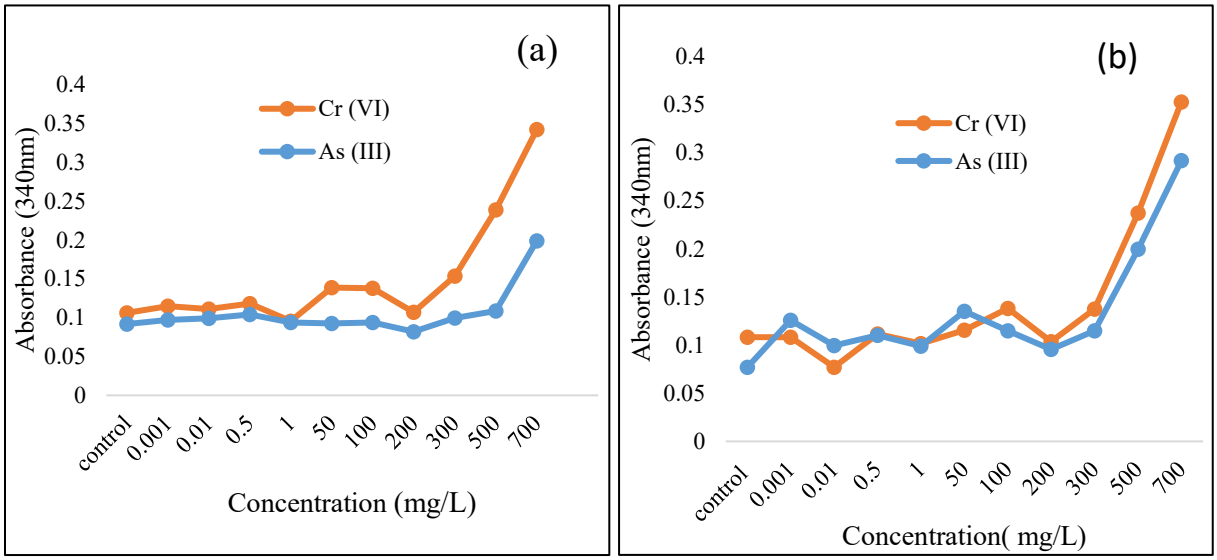


Figure: 4.4 (a) UV-Vis spectroscopy of the LDH enzyme absorbance in bacterial cells, (a) via E. *coli*, (b) via *B. subtilis*.

Growth kinetics of bacterial cells

The isolated bacterial cultures were subjected to check their growth kinetics rate at different selected concentrations of the heavy metals for 7 days. The details discussed previously in methodology. The absorbance at 600nm of bacterial isolates was recorded at regular intervals via spectrophotometer. The decline in the growth was observed at all the concentrations of heavy metals after a specific time but the greater decline was recorded at 500mg/L concentration of both heavy metals in the case of *E. coli*. Moreover, the Cr (VI) showed a more toxic effect at 500mg/L than that of As (III). However, in case of *B. subtilis*, a decline in the bacterial growth was observed

at all the concentrations but it was more specific to higher concentrations and significantly in treatment with Cr (VI) as compared to As (III).

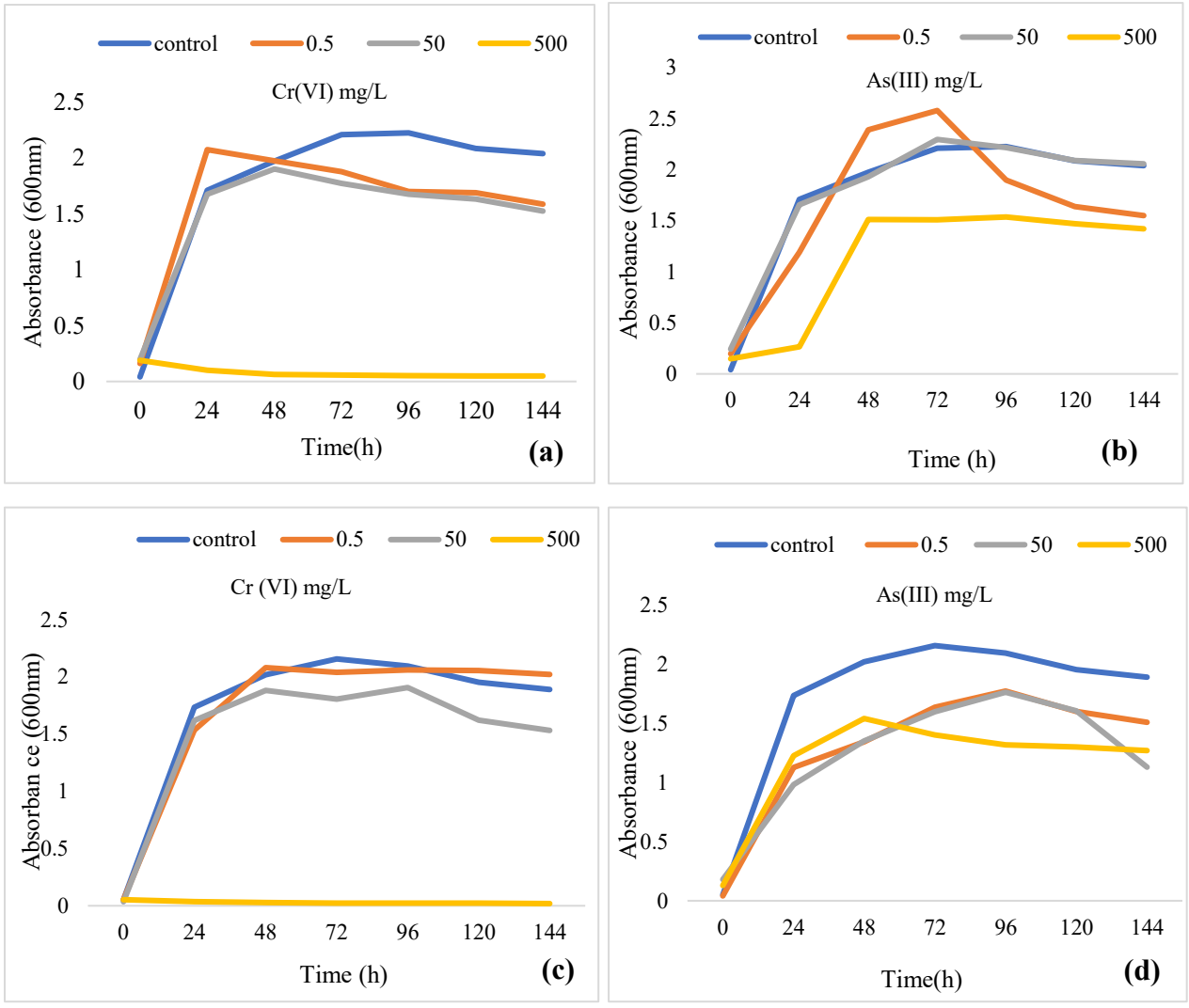


Figure: 4.5 UV- Vis spectroscopy of the effect of heavy metals on the growth kinetics of isolated bacterial strains at specific concentrations. (a) effect of Cr (VI) on *E. coli*, (b) effect of As (III) on *E. coli*, (c) effect of Cr (VI) on *B. subtilis*, (d) effect of As (III) on *B. subtilis*.

FTIR analysis of bacterial biosorption of heavy metals

Heavy metal's biosorption via *B. subtilis*

The FTIR spectra of *B. subtilis* treated with the heavy metals were compared with the control samples for the investigation of functional groups involved in the metal-microbial interaction. The bands of the spectra treated with the heavy metals demonstrated almost negligible variation from the control sample in case of *B. subtilis.* the peaks were observed at 3271.93 shifted to 3271.90 and 3263 for Cr (VI) and As (III) respectively, representing the O-H stretching of carboxylic acid group. Similarly, the peaks at 2144.58 altered to 2100 showing N=C=S stretching for Cr (VI) and 2144.71 indicating the stretching of S-C≡N for As (III). The stretching of C=C was observed at 1635cm^{-1} in the case of both heavy metals. In the same sequence the bending of N-H corresponding to amine group was also observed in both heavy metals treated spectra. In basic terms Chromate binding essentially occurs between functional groups on the cellular surface, which include the carboxyl group, hydroxyl, and phosphate groups(Zakaria, Zakaria, Surif, & Ahmad, 2007), and the functional groups involved in the Arsenite binding to bacterial cells are hydroxyl, amide and amine groups(Vishnoi, Dixit, & Singh, 2014). The peaks of chromium are more prominent in the graph than that of Arsenic because the bacteria exhibit better biosorption of Cr (VI) while in case of As (III) the stretching are almost similar with control or may be negligible which indicates that the bacteria exhibited may be some other mechanisms i.e. bioaccumulation, precipitation, or sorption.

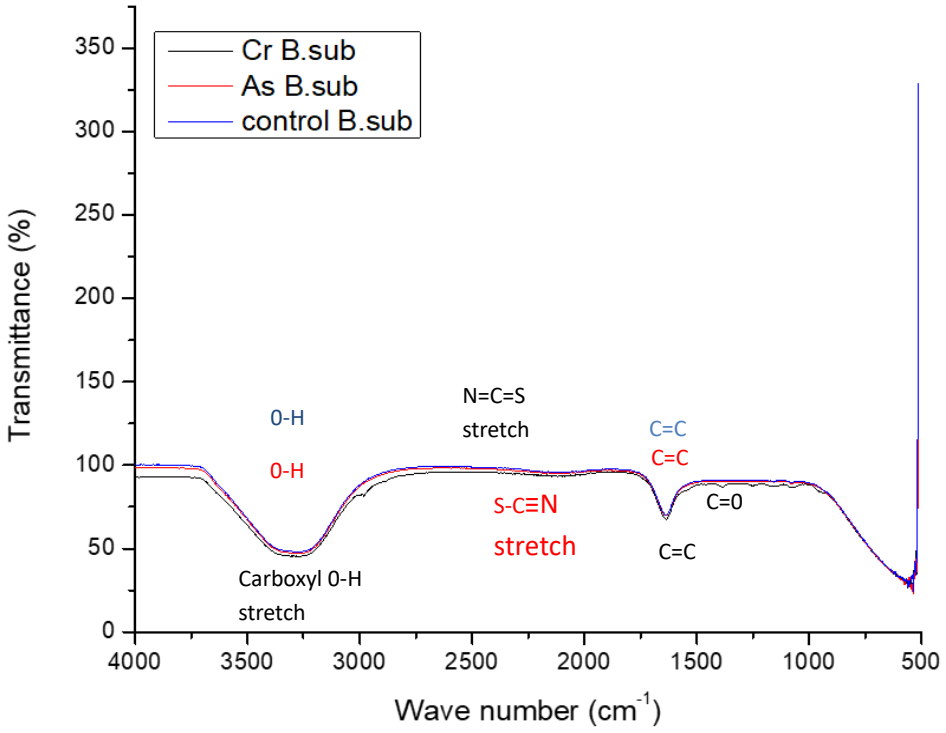


Figure 4.6 FTIR spectra for heavy metals' biosorption via *B. subtilis*

Phytotoxicity of heavy metals on the plant growth

In order to determine the effect of heavy metals on the growth of plants, the selected plants were treated with varying concentrations of heavy metals prepared from syringe filtered stock solutions, already discussed in methodology along with the heavy metal free control samples. The 1 µg/L, 10 µg/L, 500 µg/L, 1000 µg/L, 50mg/L, 100 mg/L, 200 mg/L, 300 mg/L, 500 mg/L, 700 mg/L, concentrations were employed to soak the plates having plants seeds placed on the filter paper and observed for seven days for the growth of radicles and plumules under light source. The seeds of the plants were monitored on a daily basis by adding the respective concentration of heavy metals to prevent the seeds from drying off. After seven days the influence of heavy metal concentrations was assessed by the degree of seed germination, development of the radicle, assessment of plumule length as well as the dry biomass weight compared to the heavy metal- free control sample which were

grown in the absence of contaminants. The toxic effects of Cr and As were then compared for radicle length, plumule length using the same range of heavy metal concentration.

Effect of heavy metals on *Solanum lycopersicum* (S.L) and *Eruca sativa* (E.S)

Effect on the seed germination

The determination of the toxic effects of Cr (VI) and As (III) on the development of *Solanum lycopersicum* (Tomato), and *Eruca sativa* plants was checked by the degree of seed germination compared with the control sample. Dependent on the concentration of respective heavy metal ion, the degree of seed germination ranged between 40-60% for *solanum lycopersicum* treated with the Cr (VI). The lowest degree was observed at 700mg/L concentration of chromium i.e. 0% and the highest degree was observed at 1 µg/L, 500 µg/L and 1000 µg/L, i.e. 60%. the toxic effect of Arsenic (III) was also observed on the same plant. according to which the seed germination degree of *solanum lycopersicum* was ranged between 0-20% treated with the As (III) metal concentrations. The lowest degree was observed at almost all the selected concentrations of mg/L i.e. 0% and the highest germination was observed at 500 µg/L i.e. 80%. The seed germination degree of *solanum lycopersicum* treated with the heavy metals is shown in the fig 4.7 the above discussed procedure was also applied For calculating the seed germination degree of *Eruca sativa* plant. The degree of seed germination was ranged between 40 -60% treated with the Cr (VI) as it showed highest germination of 90% at 10 µg/L, while in treatment with As (III) it showed highest germination of only 70 % at lower concentrations, on the other hand higher concentration showed no growth of the plant. The seed germination degree is expressed in the fig 4.7 the comparative analysis of both heavy metals indicates that although Cr (VI) showed toxicity and germination was inhibited but As (III) showed greater Phyto toxicity to the plant i.e. *solanum lycopersicum* in comparison with the Cr (VI).

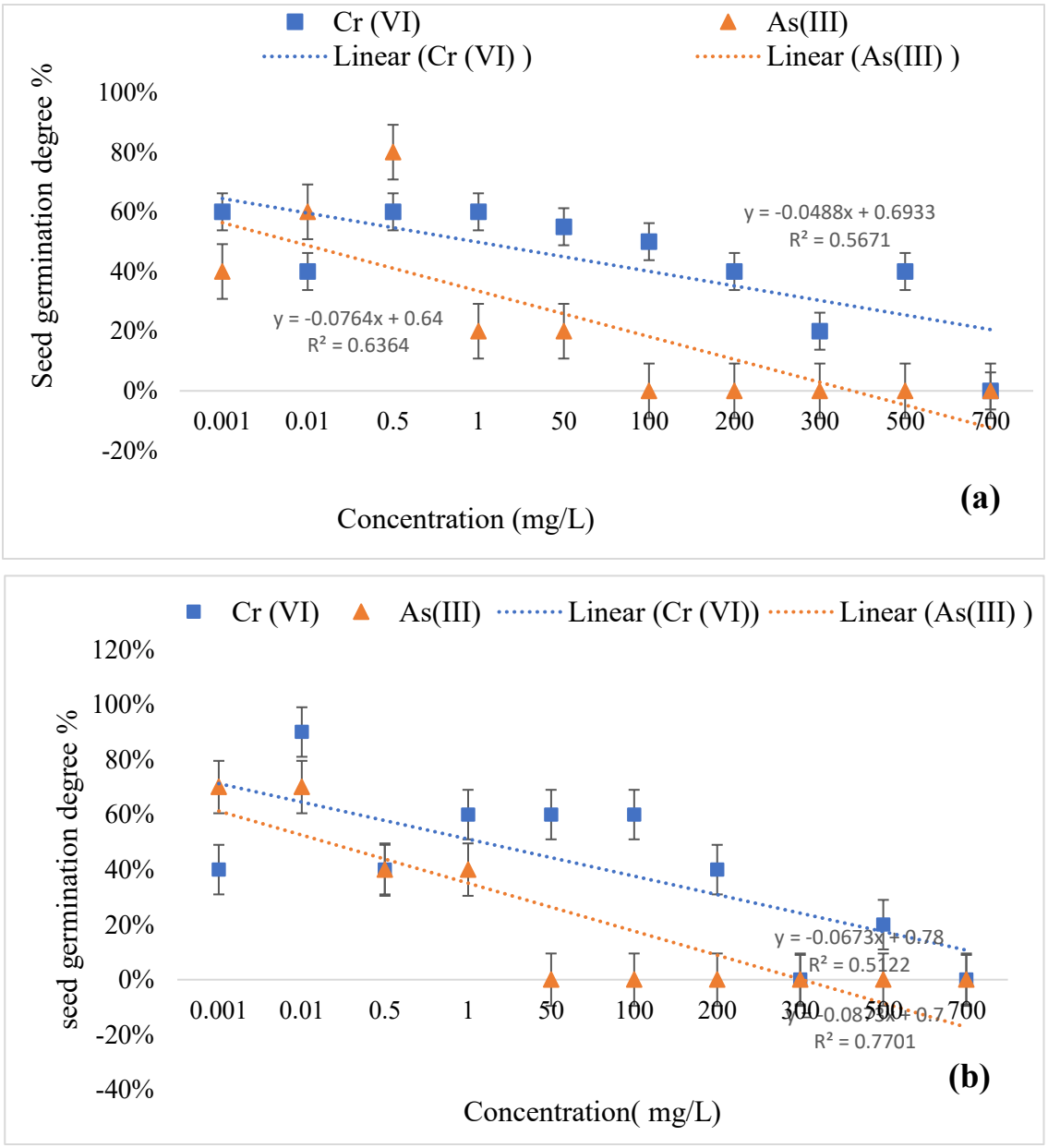


Figure 4.7 (a) Seed germination degree of Solanum lycopersicum, (b) seed germination degree of *Eruca sativa*

Effect of heavy metals on the Radicles of the plants

Similarly, the radicle length inhibition degree and plumule length inhibition degree along with the dry biomass weight inhibition percentage was also calculated from the control sample. The radicle length degree of *Solanum lycopersicum* was significantly declined with the increasing concentration of heavy metals from the control sample. The lowest inhibition for radicle length was observed in 1 µg/L i.e. 0% inhibition, while the highest inhibition degree was observed in 700mg/L i.e. 100% inhibition concentrations in lycopersicum plant treated with the Cr (VI). However, the same plant treated with the same range of concentrations of As (III) showed different behavior, in this case the lowest concentration of 10 µg/L showed 33% inhibition while the highest concentration of 700 mg/L showed 100 % inhibition. however, both the heavy metals showed almost equal toxic behavior to the radicle length of *Eruca sativa* plant i.e. highest inhibition of 100% and lowest inhibition of 43% was observed in treatment with Cr (VI), however, with As (III) the lowest inhibition rate observed was of 48 %

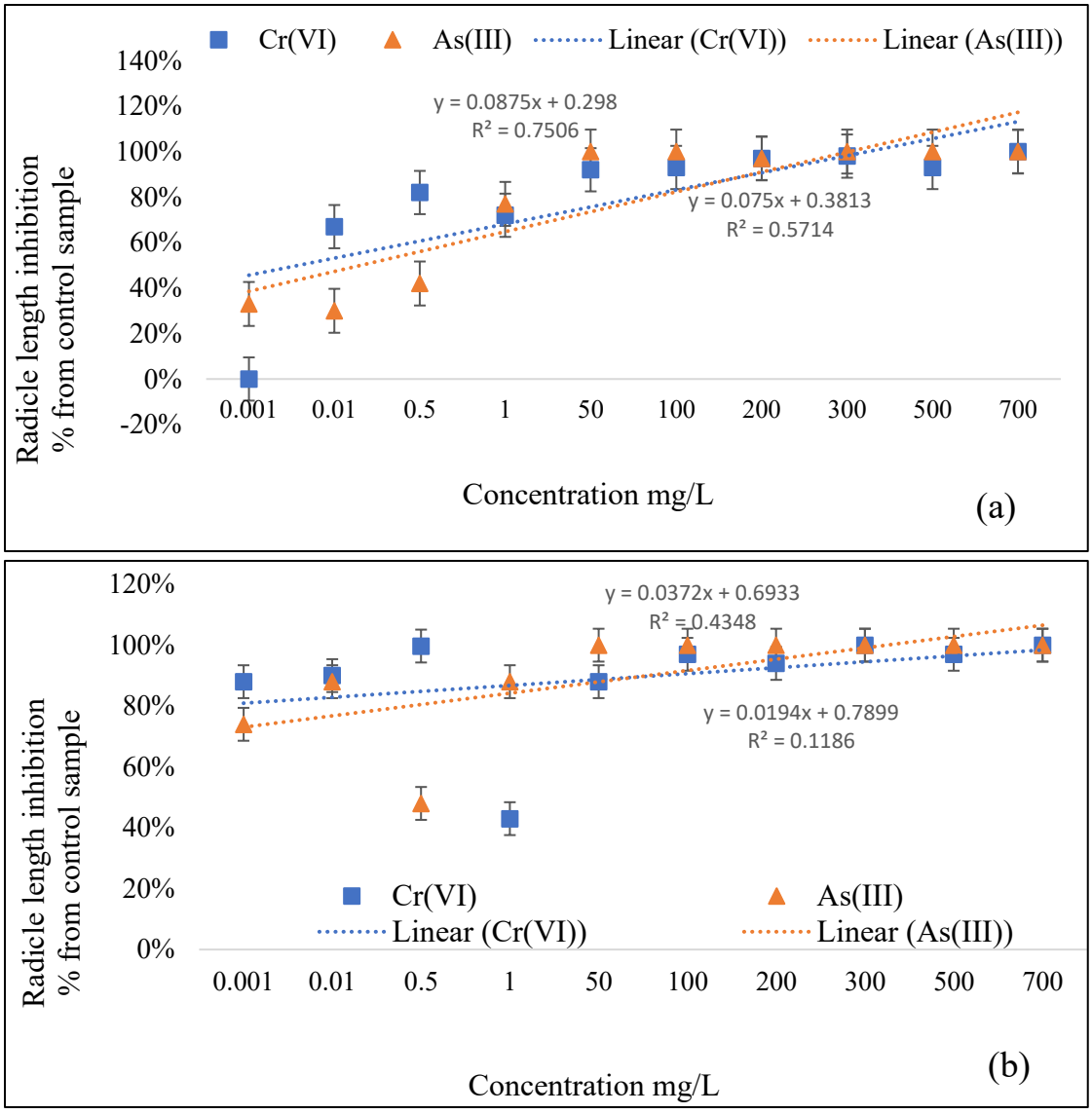


Figure: 4.8 Effects of heavy metals on the radicle length inhibition treated with heavy metals, % from control sample (a) *solanum lycopersicum*, (b) *Eruca sativa*

Effect of heavy metals on the Plumule length of the plants

Same procedure was applied for the plumule length of the same plant treated with the Cr (VI) which showed lowest inhibition of 24% at 1 µg/L, while highest inhibition of 100% at 700mg/L was observed. In case of treatment with As (III) concentrations the lowest inhibition of 31 % was observed at 500 µg/L, while the highest inhibition of 100% was observed in all the higher concentrations of mg/L of As (III). Similarly, for *Eruca sativa* plant the lowest inhibition observed was of 59% treated with Cr (VI) and for As (III) lowest

inhibition rate was of 51 %. this behavior indicates the toxic effect of both heavy metals on the plant is almost the same.

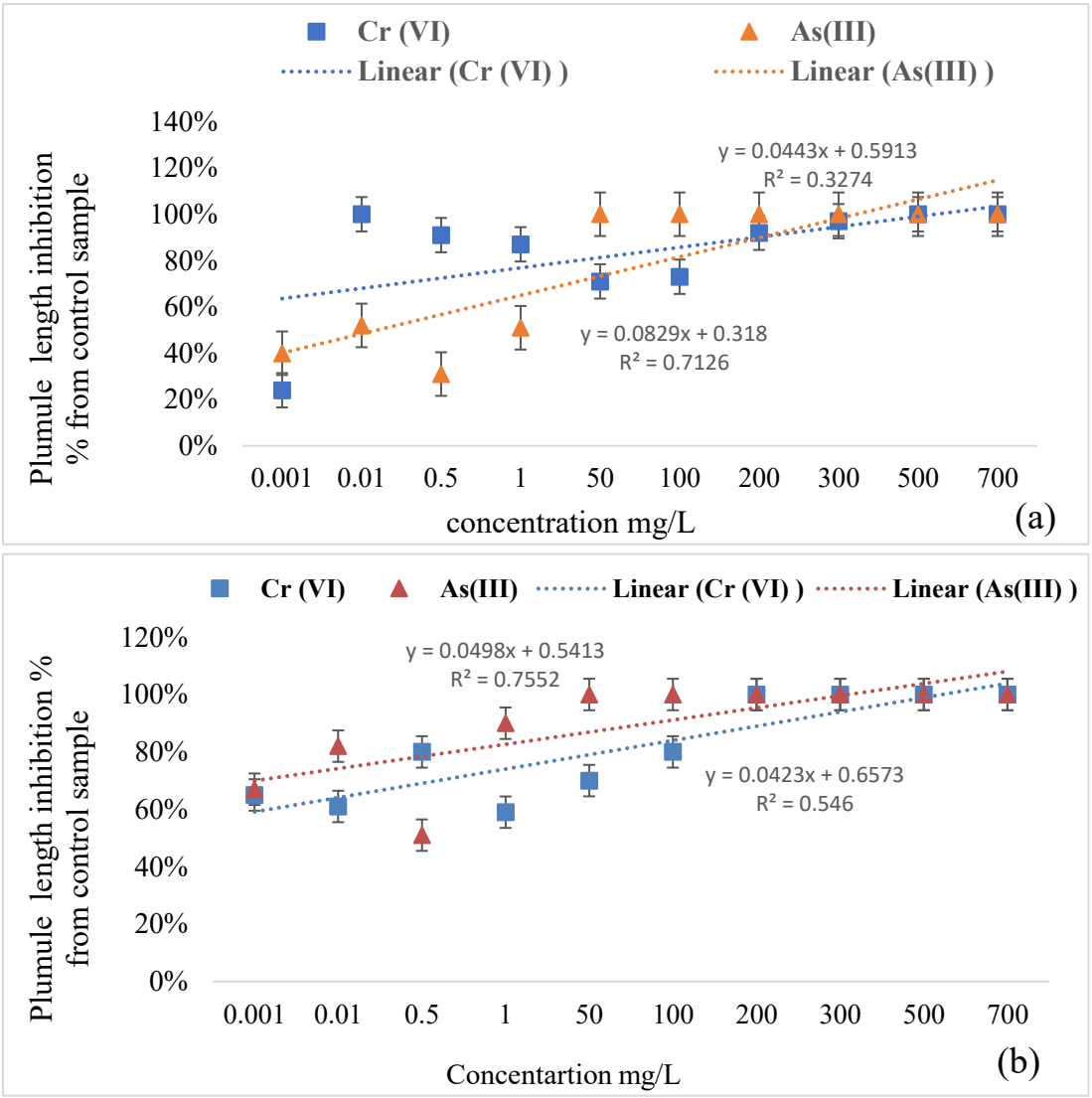


Figure 4.9 (a) Plumule length inhibition % from control sample of *solanum lycopersicum*, (b) plumule length inhibition % from control sample of *Eruca sativa*

Effect of heavy metals on the Dry biomass of the plants

The dry biomass weight inhibition degree was also calculated from the control sample and showed almost similar results in comparison with radicle and plumule inhibition degree for both plants Although some of the seeds of *Eruca sativa* showed growth but it was only to some extent i.e. on the lower concentrations while the higher concentrations of both heavy metals inhibit the growth of the plant which indicates that this plant expressed intolerance to the heavy metals and increased in weight inhibition.

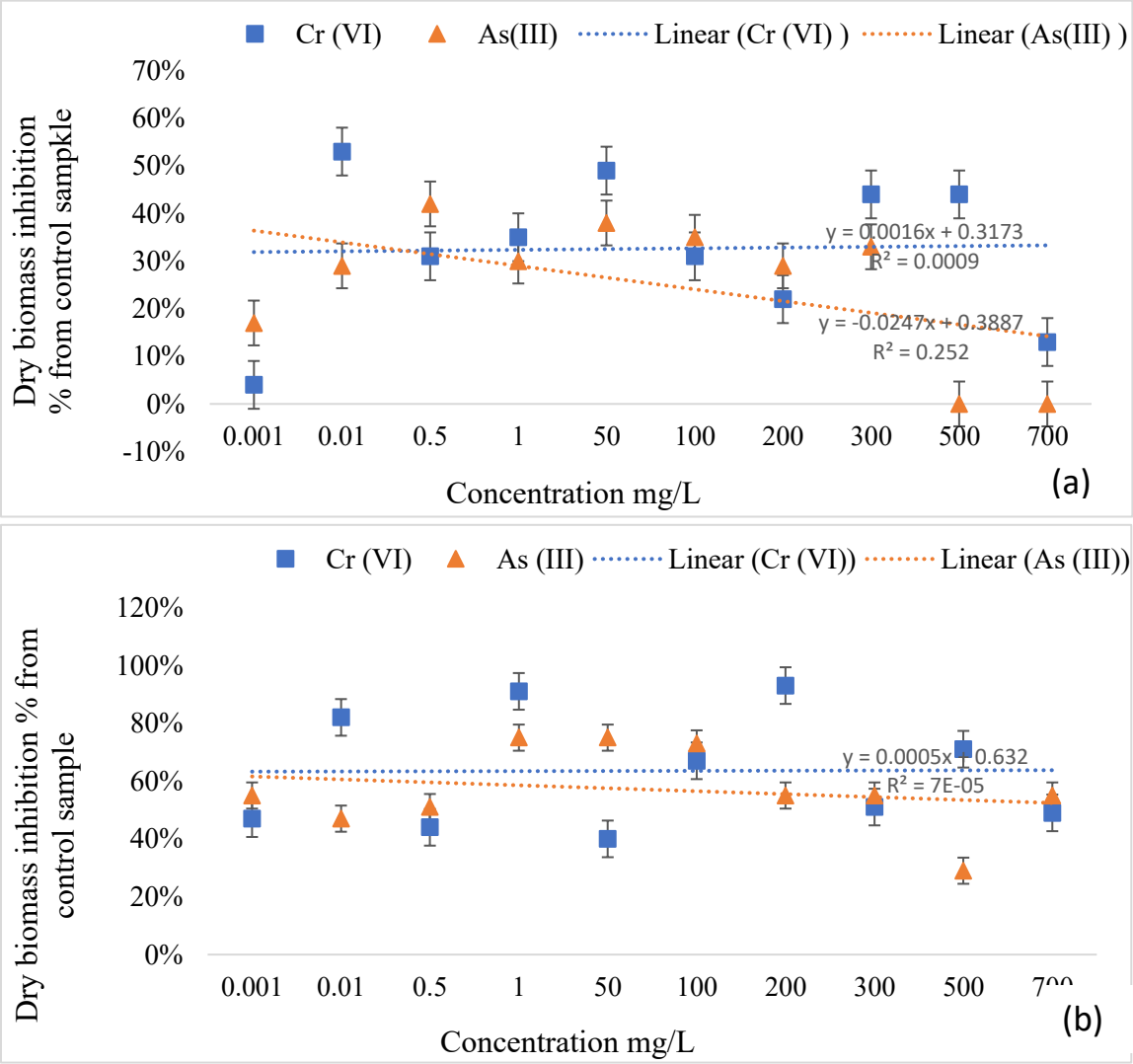


Figure 4.10 (a) Dry biomass weight inhibition treated with heavy metals % from control sample, (a) *Solanum lycopersicum*, (b) *Eruca sativa.*

The comparison elucidate that As (III) is more toxic than Cr (VI) Although, higher concentrations of the chromium are toxic to the plants, showed some tolerance to Cr(VI) at some extent on lower concentrations but treatment of the plant with As (III) showed intolerance as this heavy metal is more toxic in comparison with chromium but also showed growth of the plants at lower concentrations. The seed germination degree, radicle length, plumule length and dry biomass weight inhibition degree are expressed for both plants treated with the heavy metals expressed in the following tables, 4.1 and 4.2.

Table 4.1 Effect of Chromium (VI) on the overall growth of *solanum lycopersicum* and *Eruca sativa* plants

Concentration (mg/L)	Plant name	Radicle length(cm)	Plumule length(cm)	Dry weight (g)	Degree of seed germination %
Distilled water (control)	*Solanum lycopersicum* (Tomato)	6	7.5, 2 leaves	0.0045	100%
	Eruca sativa (Taramira)	3.5	5.1, 4 leaves	0.0020	100%
0.001	*Solanum lycopersicum*	9.1	5.7, 2 leaves	0.0043	60%
	Eruca sativa	0.4	1.8, 2 leaves	0.0024	40%
0.01	*Solanum lycopersicum*	spouting	No growth	0.0020	40%
	Eruca sativa	3.2	2, 2 leaves	0.0008	90%s
0.5	*Solanum lycopersicum*	1.1	0.7	0.0030	20%
	Eruca sativa	0.01	1, 2 leaves	0.0025	40%
1	*Solanum lycopersicum*	3	1	0.0029	20%
	Eruca sativa	2	2.1, 3 leaves	0.0004	60%
50	*Solanum lycopersicum*	0.5	2.2, 2 leaves	0.0023	40%
	Eruca sativa	0.4	1.5, 3 leaves	0.0012	60%
100	*Solanum lycopersicum*	0.4	2	0.0030	60%
	Eruca sativa	0.1	1, 2 leaves	0.0009(0.0015)	60%
200	*Solanum lycopersicum*	0.2	0.6	0.0035	40%
	Eruca sativa	0.2	0	0.0003	40%
300	*Solanum lycopersicum*	0.1	0.2	0.0025	20%
	Eruca sativa	No growth	No growth	0.0022	0%
500	*Solanum lycopersicum*	0.4	0	0.0025	40%
	Eruca sativa	0.1	0	0.0013	20%
700	*Solanum lycopersicum*	No growth	No growth	0.0039	0%
	Eruca sativa	No growth	No growth	0.0023	0%

Table 4.2 Effect of Arsenic (III) on the overall growth of *solanum lycopersicum* and *Eruca sativa* plants

Concentration (mg/L)	Plant name	Radicle length(cm)	Plumule length(cm)	Dry weight (g)	Degree of seed germination %
0.001	*Solanum lycopersicum*	4	4.5, 2 leaves	0.0012	40%
	Eruca sativa	0.9	1.7, 4 leaves	0.0020	70%
0.01	*Solanum lycopersicum*	6.5	3.6, 2 leaves	0.0032	60%
	Eruca sativa	0.4	0.9	0.0024	70%
0.5	*Solanum lycopersicum*	3.5	5.2, 2 leaves	0.0026	80%
	Eruca sativa	1.8	2.5, 4 leaves	0.0022	40%
1	*Solanum lycopersicum*	1.4	3.7	0.0043	20%
	Eruca sativa	0.4	0.5	0.0011	40%
50	*Solanum lycopersicum*	No growth	No growth	0.0028	0%
	Eruca sativa	No growth	No growth	0.0011	0%
100	*Solanum lycopersicum*	No growth	No growth	0.0029	0%
	Eruca sativa	No growth	No growth	0.0012	0%
200	*Solanum lycopersicum*	0.2	0	0.0032	20%
	Eruca sativa	No growth	No growth	0.0020	0%
300	*Solanum lycopersicum*	No growth	No growth	0.0030	0%
	Eruca sativa	No growth	No growth	0.0020	0%
500	*Solanum lycopersicum*	No growth	No growth	0.0047	0%
	Eruca sativa	No growth	No growth	0.0032	0%
700	*Solanum lycopersicum*	No growth	No growth	0.0050	0%
	Eruca sativa	No growth	No growth	0.0020	0%

Figure: 4.11 Growing plant seeds treated with the different concentrations of heavy metals

FTIR analysis of heavy metals' biosorption by plants

Biosorption of Cr (VI)

Figure 4.12 presents the findings of the FTIR spectra of the biomass of plants subjected to treatment with the heavy metals As (III) and Chromium (VI). Both the *solanum lycopersicum* and *Eruca sativa* plants demonstrated remarkably similar maxima of Cr (VI) across the infrared spectra. The bands were observed at (3282.74cm-1 and 3275.19) for S.L and E.S respectively which represents the strong binding of Cr (VI) and stretching of hydroxyl group (O-H) as were in the control. According to reports, the diversity in wave number values of hydroxyl stretching frequencies is caused by the presence of metal conjunction points on seaweeds, which comprise hydroxyl, carbonyl, and ether groups with cations in a variety of multiple patterns(Chen & Yang, 2006). Similarly, The bands located at 2923.88 and 2924.11 cm-1 demonstrated a noteworthy contribution from the C-H

stretching band to the coupling of Chromium (VI) to S.L. and E.S respectively. Comparing with the previous investigation by(Sheeja, christobel, & Lipton, 2016) the same bands were observed for G. corticata and S. wightii treated with the Cr (VI) heavy metal(Sheeja et al., 2016). The peaks at 1635.22 and 1634.76 representing the bending of N-H group in both respective plants as compared to control, indicating the binding of Cr (VI).

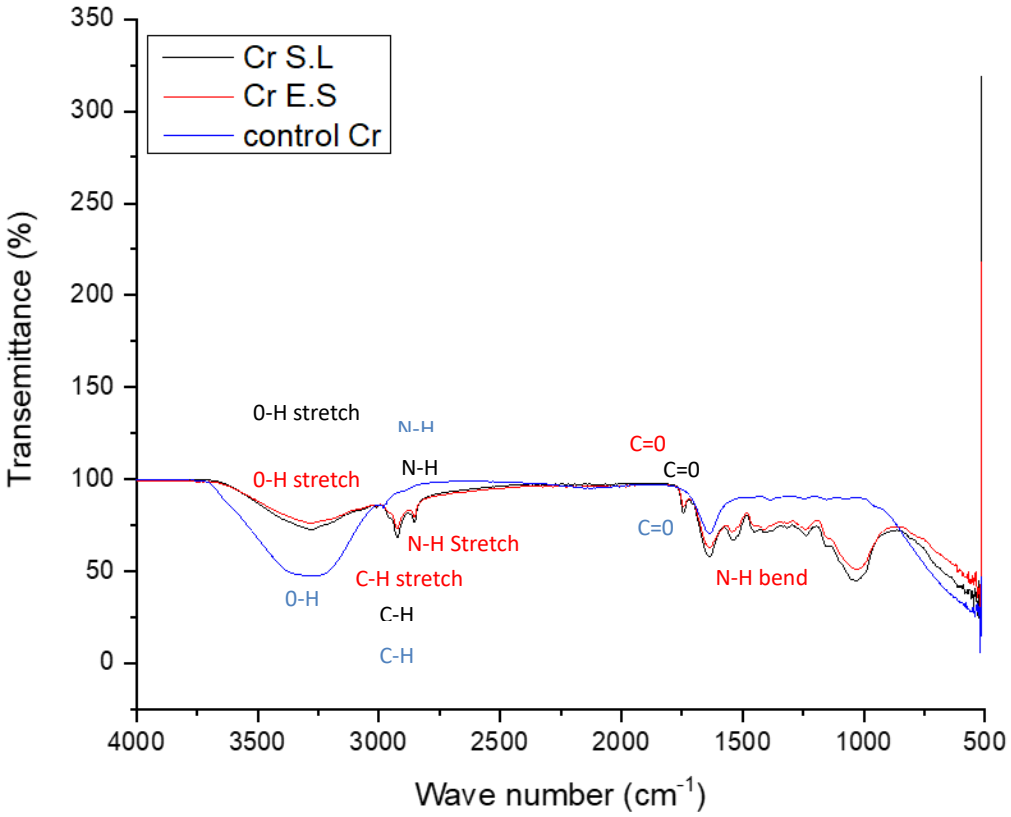


Figure 4.12 FTIR spectra for Cr (VI) biosorption via *Solanum lycopersicum* (S.L) and *Eruca sativa* (E.S).

Biosorption of As (III)

The investigation of the FTIR spectra of As (III) biosorption revealed that the cell framework of biosorbent plants contained amino, carboxylic, hydroxyl, and carbonyl groups in them. Exhibiting wide O–H stretch carboxylic bands in the 3,276.61 and 3281.73

cm−1 domains for S.L. and E.S., respectively. leading to the amines' NH_2 asymmetric stretching phase at 2923.04 and 2921.80 for correspondingly S.L and E.S plants. whereas C=O chelation stretching of the carboxylic group caused the carboxyl/phenolic straining bands with directing of NH_3 + symmetric across the 2,927 and 2,369 cm−1 areas to be seen by (Yun, Park, Park, & Volesky, 2001). The peak patterns that show up in the regions of 1,635.22 and 1641.29 cm−1 for S.L and E.S respectively, could be related to asymmetric stretching of C=C and C=O, conjugated to an NH bending manner, and suggest the presence of an amide I band. According to(Nigam, Gopal, & Vankar, 2013) the same stretching of >C=N, >C=C and C=O were observed at the region of 1,635 cm^{-1} of wavelength for As (III) biosorption by *Hydrilla verticilata*.

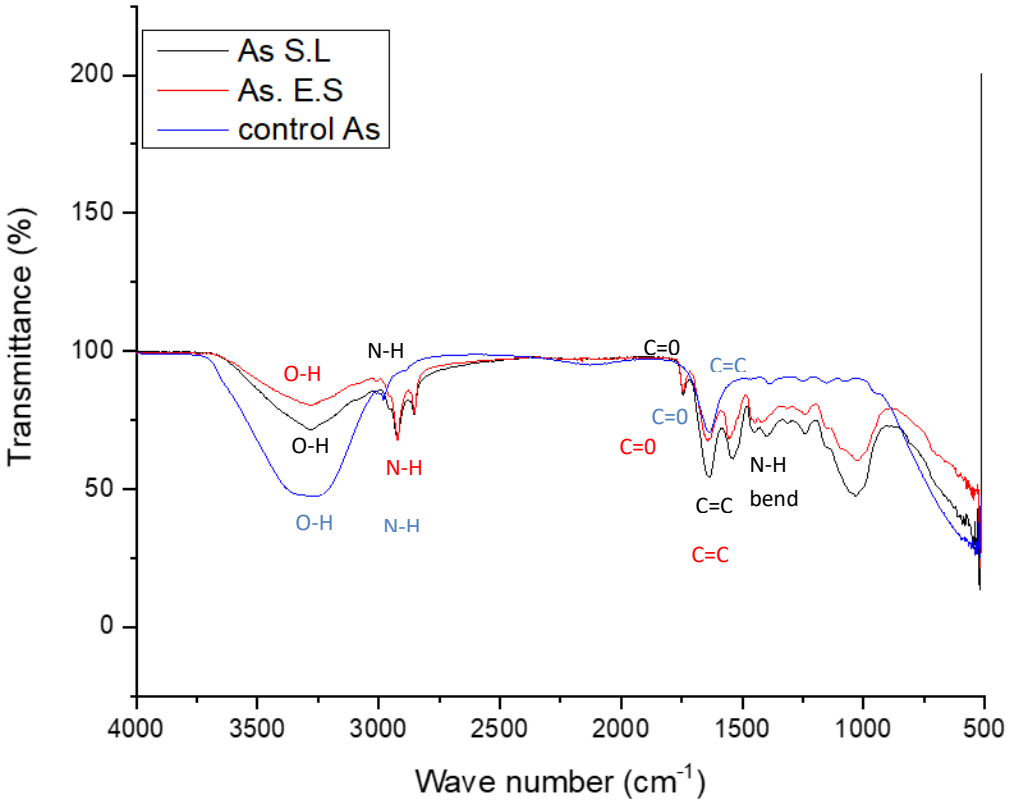


Figure 4.13 FTIR spectra for As (III) biosorption via *Solanum lycopersicum* (S.L) and *Eruca sativa* (E.S).

Lethality assay for *Artemia salina* (brine shrimp)

The activity of artemia salina (brine shrimp) was analyzed by treatment with the varying concentration of heavy metals prepared from the above-mentioned syringe filtered stock solutions. the nauplii were released by the hatching of eggs in the saline solution after 48 hrs. of continuous aeration. After 48 hrs. the nauplii were exposed with the varying concentrations of heavy metals. i.e. Cr (VI) and As (III), prepared in the test tubes, having 20 number of cells in each. After the exposure of cells to the heavy metals they were observed for their death rate after every ten minutes. As the heavy metals used in the experiment were already very toxic, that's why they were observed for their toxic behavior in time intervals. In addition, due to the greater cytotoxicity of heavy metals even at concentrations of micrograms, some additional concentrations were also employed including 2 µg/L, 4 µg/L, 6 µg/L, 8 µg/L, 250 µg/L, 750 µg/L. The effect of heavy metals on the activity of brine shrimps were then compared simultaneously at three intervals i.e. the time rate at which nauplii started dying, the time rate at which 50% of the population killed, and the time interval at which almost all the population of nauplii were killed completely the complete death of all the cells were observed after 2hrs, 10 minutes at 700mg/L concentration while at the lower concentration of 1 µg/L the complete death observed after 4hrs 50 min, treated with the Arsenic (III). However, at 700 mg /L concentration of Cr (VI) the death of the nauplii was observed after short duration of 1hr, while at lower concentration of 1 µg/L, it was after 3hrs 50 min observed. This behavior indicates in this study of *Artemia salina* the chromium (VI) heavy metal showed greater cytotoxic behavior as compared to Arsenic (III).

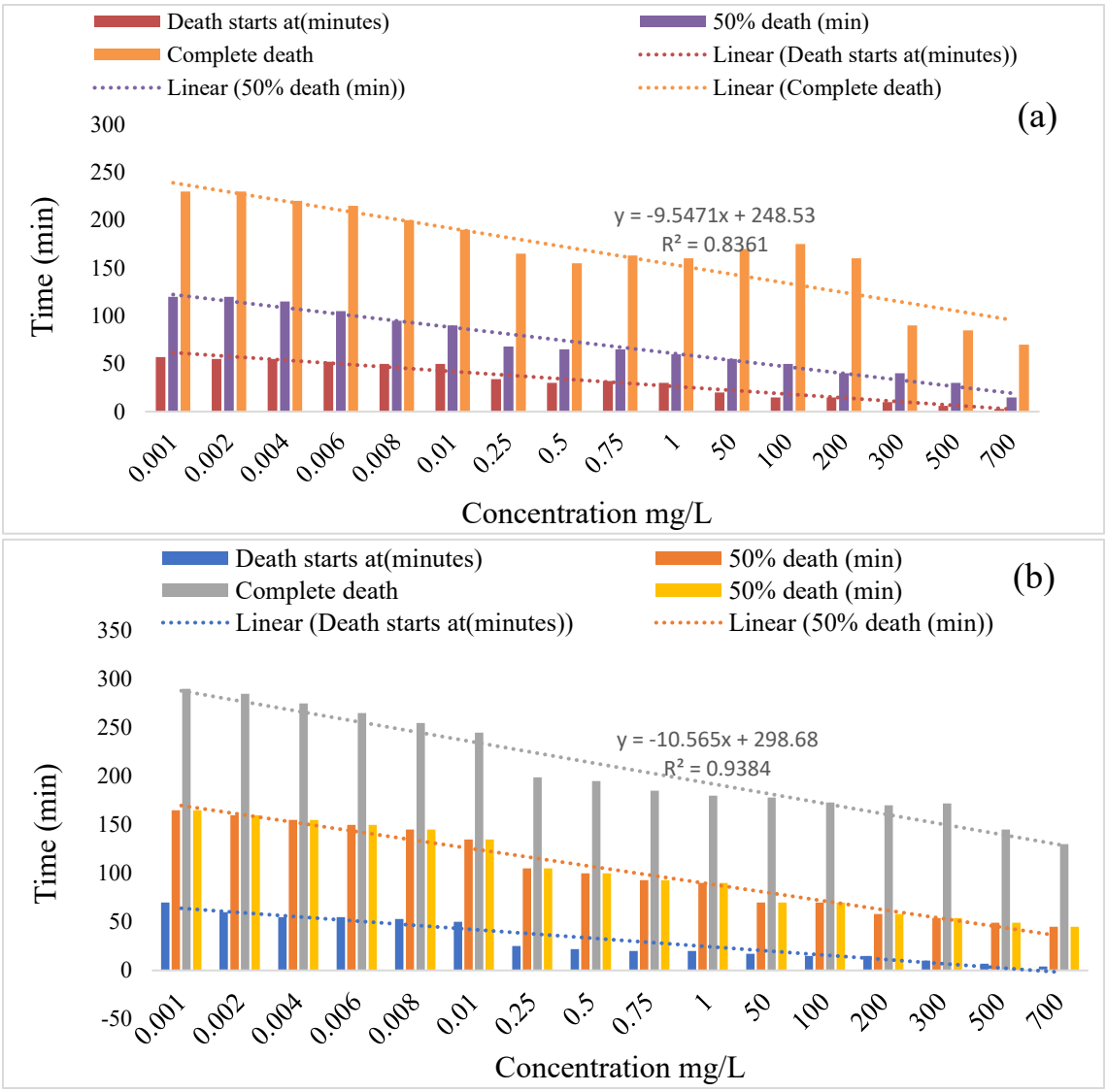


Figure 4.14 (a) Cytotoxic Effects of chromium (VI) on the activity of *artemia salina*, (b) cytotoxic effects of Arsenic (III) on the activity of *Artemia salina*.

Discussion

Environmental concerns regarding heavy metal toxicology are increasingly important. Certain heavy metals are released into the environment via scrap from industries, where they have long-lasting detrimental effects on both human beings and the ecosystem (Pujari & Kapoor, 2021). The excessive compaction *of harmful heavy metals* has an adverse effect on *soil* microbes, which are vital to the global cycling of nutrients, the decomposition of plant and animal waste, and the reconstitution of nutrients. Certain heavy metals, including mercury, arsenic, thallium, chromium, cadmium, lead, and cadmium, have no biological function. But because of their environmental existence, they will unavoidably find their way into the human body(V. Kumar et al., 2023). The excessive build- up of toxic heavy metals also negatively affects soil microbes, which are vital to the global cycling of nutrients, the decomposition of plant and animal waste, and the reconstitution of nutritional components.

Chromium is heavily used in a wide range of industries, such as metallurgy, leather tanning, pigment, mining, electroplating, corrosion mitigation, and electronic and electrical equipment. Research has shown that hexavalent chromium is a formidable carcinogen and that the exposure of chromium due to emissions from industry is widespread in the environment(DesMarias & Costa, 2019). The most prevalent sources of ingestion of inorganic arsenic, which is deadly form of arsenic, are contaminated food and water. Contamination contributors involve wood preservatives, insecticides, lead and copper smelting operations, and erupting volcanoes(Muzaffar, Khan, Srivastava, Gorbatyuk, & Athar, 2023). In the investigation that subsequently followed, the effects of heavy metals, such as Cr (VI) and As (III) (a herbicide), on bacteria (*B. subtilis* and *E. coli*), were evaluated using differential toxicological analysis. And to determine these bacterial strains' aptitude to transform these heavy metals biologically. Moreover, the toxicological behavior of heavy metals was also investigated against plants (*Solanum lycopersicum*, and *Eruca sativa*) and animals (*Artemia salina).*

In the present investigation, two gram-positive and gram-negative bacterial strains, *B. subtilis* and *E. coli*, were subjected to different doses of the heavy metals Cr (VI) and As (III). There were notable variations in the growth responses of *B. subtilis* and *E. coli* when exposed to varying concentrations of Cr (VI) and As(III). Each of the strains grew slowly at first and then substantially progressively as the intervals of incubation were extended. The bacteria proved capable of surviving better at reduced concentrations. However, a maximally deadly effect was observed on the bacteria's development as the concentration was raised to double and triple treatment rate. Compared to all other concentrations, 500 and 700 mg/L were particularly cytotoxic to bacterial cells and significantly inhibited microbial growth. The *E. coli* demonstrated more sensitivity to the As (III) heavy metal than that of the chromium (VI) as compared to *B. subtilis,* which showed more sensitivity to the chromium. The harmful effects of heavy metals i.e. Cr(VI) and As(III) could be attributable to these heavy metals' increased solubility and mobility in an aqueous environment compared to solid medium, according to another experiment carried out while cultivating bacterial cells in a liquid culture medium(Su et al., 2018), (Çelebi, Gök, & Gök, 2020). The enhanced heavy metal ion transport across bacterial membranes may possibly be the cause of the growth inhibition seen following exposure to heavy metals. After breaking through the membrane, heavy metals are likely to interfere with the metabolic processes of bacteria, potentially causing changes in growth or fatal consequences. Furthermore, the bacterial strain may occasionally die as a result of accumulation of heavy metals by bacterial cells and the interruption of respiration during growth underneath the stress caused by heavy metals. Since *B. subtilis* and *E. coli* are regarded as components of the gut microbiota. Therefore, previous studies have shown that these heavy metals, both alone and in combination, have comparable detrimental effects on the development of gut microbial species exposed to dosages of As (iii) and Cr (vi) heavy metals(D. P. Singh et al., 2023).

The current investigation employed the capability of particular bacterial strains, *B. subtilis* and *E. coli*, to ascertain the bioremoval of Cr (VI) and As (III) using spectrophotometric analysis at all the designated concentrations. Within a period of 48 hours, an effective bioremoval percentage of 98% for Cr (VI) and 96% for As (III) via *E. coli* was attained. However, within 48 hours, *B. subtilis* induces 83% of Cr (VI) and 98% of As (III) to be bio transformed. These outcomes exceeded the prior reported by (Ramli et al., 2023) Chromium (VI) percentages by a substantial margin. which were 82% and 41% for gram positive *Streptomyces werraensis* and gram-negative *Pseudomonas sp*. after 7 and 3 days respectively. additionally , A prior investigation by (X. Wang et al., 2020) indicated that Acidithiobacillus ferrooxidans removed 95% of As (III). which is less than the findings of the present study.

Lactate dehydrogenase (LDH) enzyme analysis was employed for determining the toxicity that heavy metals brought to bacterial cells. The enzyme lactate dehydrogenase is often employed as a stress biomarker to recognize tissue damage with prolonged exposure to xenobiotics, such as hazardous heavy metals(Alonso-Bernáldez et al., 2023). Consequently, the assessment of lactate dehydrogenase activity was conducted with respect to varying concentrations of heavy metals. The bacterial membrane was damaged by all concentrations of Cr (VI) and As (III) heavy metals, but the greatest doses of Cr (VI) significantly affected both microbial strains of *B. subtilis* and *E. coli* at higher concentrations of 500 and 700mg/L. a prior study also revealed the higher toxicity of Cr (VI) after being absorbed by the cell, Cr (VI) interacts with DNA-protein complexes to produce DNA-DNA cross links, which may eventually have deleterious and carcinogenic effects. ROS is produced by chromium, which can also lead to the peroxidation of lipids and increase the quantity of LDH released(de Moura Sousa, Moreira, Cardoso, & Batista, 2023).

In the present research both isolated bacterial strains were also subjected to check their growth kinetics response to different applied concentrations of heavy metals in comparison with the untreated one. Three concentrations of 500µg/L, 50 mg/L, 500mg/L were employed. In the current study Optimal levels of tolerance to varying heavy metal concentrations were demonstrated by *E. Coli* treated with As (III). Growing slowly at first,

the bacterial strain expanded its growth phase, however as its time period accelerated, its growth rate grew linearly. Bacterial cells exhibited maximal viability and a modest decline in growth when compared to control at lower concentrations of Cr (VI) and As (III). Nevertheless, when compared to the normal rates of administration of 500 µg/L and 50 mg/L for both heavy metals, the higher concentration of 500 mg/L administered exhibited the most detrimental effect on *E. coli* proliferation. As demonstrated in another investigation(Tanu, Hakim, & Hoque, 2016), the elevated solubility and fluidity of other heavy metals may be the reason for the deleterious impacts of heavy metals including Cr (VI) while cultivating a bacterial strain in Luria broth polluted with heavy metals. This might contribute to the growth reduction observed in the present research, possibly because of heavy metal-containing inorganic ions being absorbed and transported across cell membranes. Additionally, heavy metals have been demonstrated identified in earlier research that they slow down the growth kinetics of isolates of bacterial consortium, from the northwest Antarctic Peninsula soil when they are cultivated in nutrient broth media that also contains heavy metals including As(III)(Tengku-Mazuki et al., 2023).

On the other hand, in recent investigation *B. subtilis* treated with the lower concentration of 500 µg/L demonstrated almost equal growth compared to the untreated one (control) in case of both heavy metals. But higher concentration(500mg/L) of Cr (VI) illustrated detrimental effects on the growth kinetics of the bacteria. This discovery aligns with prior descriptions of As (III) resistance in Gram-positive bacteria. In this regard, phosphate transporters and aquaglycoporins are primarily responsible for the absorption of arsenate and Arsenite by bacteria. Arsenic undergoes metabolism (such as reduction, oxidation, methylation, etc.) by arsenic resistance efflux system after invading bacterial cells(Kabiraj, Biswas, Halder, & Bandopadhyay, 2022) (Silver, 1996).

The current study additionally evaluated the phytotoxicity of arsenic and chromium for the plants that include seeds of *Eruca sativa* and *Solanum lycopersicum*. The effect of heavy metals on the seed germination of both plants was almost similar depending upon the treated concentration compared to the control. Seed germination was used to evaluate the effects of Cr and As on plant development with regard to Cr (VI) tolerance. For Solanum lycopersicum, In comparison to As (III), which ranged from 0–20% the degree of

germination for Cr(VI) was between 40–60%. Depending on the concentration of heavy metal ion . A similar case was observed for *Eruca sativa* in which the plant demonstrated a little tolerance to Cr (VI) than As (III) which indicated the higher toxicity of As (III) on both Plants than Cr (VI). Parallel to this study , another investigation was conducted by (Diaconu et al., 2020) according to which Cr (VI) tolerance was higher than the other heavy metal i.e. Cd for *L. sativum* plant. Some researchers have documented a reduction of up to 45% in alfalfa seeds (*Medicago sativa L.*) germination efficacy when soaked in solutions comprising 5–40 mg Cr(VI)/L(Peralta et al., 2001).

The investigation did not yet distinguish between metal redistribution in plumules or leaves and the uptake of Cr (VI) or As (III) through radicle system. Despite the fact that As and Cr can be incorporated in both oxidation forms (As (III),(V)] and Cr(III), Cr(VI)], respectively, But It is not yet determined how each ion absorption process works(H. P. Singh, Mahajan, Kaur, Batish, & Kohli, 2013). However, an examination of the impact of heavy metals on radicles, plumule lengths and dry biomass inhibition calculated percentages from the control sample, demonstrated that, at elevated metal ion dosages, radicle and plumule lengths dramatically decreased from the control sample utilizing the identical range of Cr (VI) and As (III) ratios in aqueous solution. But the plants also showed maximum growth at lower concentrations. Yet in this case Cr treated plants indicated somehow a tolerant behavior than treatment with As. This behavior indicates the tolerance of Cr at radicle and plumule level. According to research by other people, the highest concentration of Cr is first collected in the radicles and subsequently in the leaves. and fruits, as a result of particular insoluble Cr complexes formed in radicles which lead to poor metal transmission towards plumules. Prosopis laevigata undergoing Cr(VI) exposure was found to have less secondary radicles(Buendía-González, Orozco-Villafuerte, Cruz-Sosa, Barrera-Díaz, & Vernon-Carter, 2010). Cr buildup in radicles and plumules can have additional impacts than growth modifications, like metabolic alterations: such as in (Pavel, Sobariu, Diaconu, Stătescu, & Gavrilescu, 2013) (i) modified production of pigments crucial to plant survival (anthocyanins, chlorophyll), (ii) enhanced formation of metabolic products like glutathione and ascorbic acid, and (iii) transformed metabolic pathways,

which may lead to the biosynthesis of novel substances that provide tolerance or resistance to Cr.

The detrimental consequences of heavy metals on the activity of animal cell i.e. artemia salina was also examined in the present reasearch. Time based toxicity assessment was employed in case of 48 h matured artemia salina because of the less toletrant behavior of animal cell to the selected heavy metals as both i.e. Cr (VI) and As(III) heavy metals were lethal to the brine srimps. In treatment with As (III) the 100% mortality was identified after the duration of 2hrs at highest (700mg/L) concentartion , while 4hrs. at lowest of 1 µg/L concentration. However , exposure with Cr (VI) the 100 % mortality rate was observed after a short duration of 1 hr. at highest concentration. The organism iluustrated intolerance to both heavy metals but dependent on the time period the mortality flutuate which demoscstrated the higher toxicity of Cr (VI) in comparison with As (III).A similar investigation by (Mengibar Guerrero, N. 2017),(Gajbhiye & Hirota, 1990) confers the letality of other havy metals including chromium based on the time period.

Conclusion

The present investigation evaluated the capability of bacteria to biotransform the selected metals and has supplied comprehensive data on the biological cytotoxicity of Cr (VI) and As (III) heavy metals on bacteria, plants, and aquatic animals.

1. These outcomes clearly demonstrated that different rates of heavy metals applied separately had varied effects on the capacity of the bacterial samples *B. subtilis* and *E. coli* with erratically elevated levels of heavy metals to grow, develop biofilms, thrive cells, and have permeability in their internal membranes. Additionally, when cells were exposed to high concentrations of heavy metals, it was evident that the level of released LDH enzymes had increased.

2. Moreover, the bioremoval capability for these heavy metals was ascertained in this investigation. In a shake flask incubator, bacterial specimens were evaluated for their capability to reduce heavy metals. The best results, achieved in 48 hours, were within 98 to 100% for both heavy metals produced by *E. Coli* and *B. subtilis*.

3. Likewise From the foregoing, it is clear that exposure to heavy metals significantly damages plants on both molecular as well as biochemical levels. It additionally disrupts fundamental physiological activities in plants, including the repression of general growth mechanisms. Furthermore, throughout the early phases of seedling construction, these heavy metals had an influence on diminishing and/or inhibiting the events of germination of seeds, radicle/plumule development, and numerous subsequent reproductive activities.

4. The study additionally glanced at aquatic animals and found that nauplii of Artemia Salina are exceptionally vulnerable to heavy metal toxicity. The investigation revealed that the usage of metallic materials has extended widely in residence, commercial, and agricultural settings, but it's crucial to be aware of possible detrimental impacts they may have on the natural world and public health. To reduce the adverse consequences of metals, assessment for cytotoxicity by laboratory testing ought to be conducted before applying in any sector.

Future prospects

1. In order to determine what kinds of genes or enzymes are modulated by heavy metal stress and which specific biochemical pathways contribute in the biological remediation of heavy metals, more investigation into the cellular interactions among microorganisms and metals needs to be conducted.

2. In order to comprehend the fundamental causes of metal toxicity used as insecticides and guide initiatives towards establishing more environmentally friendly, resilient agrochemical methods, additional investigation is required on the interactions between heavy metals, soil microorganisms, and plants.

3. Furthermore, it is important to comprehend the harmful effects (at the gene level) of As (III) and Cr (VI) toxicity to microorganisms, plants, and their internal structures.

4. Additionally, there seems to be a crucial demand to focus on the application of environmentally friendly, safe agricultural products related to heavy metals that have restricted side effects and to develop practical methods to lessen the detrimental effects of synthetic insecticides on soil microorganisms, profitable crops, and eventually human health.

References

Ahmed, M. F., et al. (2021). "Carcinogenic and non-carcinogenic health risk of arsenic ingestion via drinking water in Langat River Basin, Malaysia." Environmental geochemistry and health **43**: 897-914.

Alessandrello, M. and D. L. Vullo (2018). "Biotreatment of Cr (VI)-containing wastewater mediated by indigenous bacteria."

Ballav, N., et al. (2012). "High efficient removal of chromium (VI) using glycine doped polypyrrole adsorbent from aqueous solution." Chemical Engineering Journal **198**: 536-546.

Brito, E. M., et al. (2015). "Impact of hydrocarbons, PCBs and heavy metals on bacterial communities in Lerma River, Salamanca, Mexico: investigation of hydrocarbon degradation potential." Science of the total environment **521**: 1-10.

Choppala, G., et al. (2013). "Chromium contamination and its risk management in complex environmental settings." Advances in agronomy **120**: 129-172.

Hassan, M. M. (2018). Arsenic in groundwater: poisoning and risk assessment, Crc Press.

Hlihor, R. M., et al. (2014). "Recent advances in biosorption of heavy metals: support tools for biosorption equilibrium, kinetics and mechanism."

Jutsz, A. M. and A. Gnida (2015). "Mechanisms of stress avoidance and tolerance by plants used in phytoremediation of heavy metals." Archives of Environmental Protection **41**(4): 104.

Kandhol, N., et al. (2022). "Nanoparticles as a potential protective agent for arsenic toxicity alleviation in plants." Environmental Pollution **300**: 118887.

Kile, M. L., et al. (2011). "A pathway-based analysis of urinary arsenic metabolites and skin lesions." American journal of epidemiology **173**(7): 778-786.

Manu, M., et al. (2018). "The effect of heavy metals on mite communities (Acari: Gamasina) from urban parks-Bucharest, Romania." Environmental Engineering & Management Journal (EEMJ) **17**(9).

Pal, S., et al. (2017). "Bisphenol S impairs blood functions and induces cardiovascular risks in rats." Toxicology reports **4**: 560-565.

Paula, J. F., et al. (2012). "Arsenic determination in complex mining residues by ICP OES after ultrasonic extraction." Microchemical Journal **104**: 12-16.

Pavel, V. L., et al. (2013). "Symbiosis in the environment biomanagement of soils contaminated with heavy metals." Eur. J. Sci. Theol **9**(4): 211.

Sarkar, A. and B. Paul (2016). "The global menace of arsenic and its conventional remediation-A critical review." Chemosphere **158**: 37-49.

Saud, S., et al. (2022). "The impact of chromium ion stress on plant growth, developmental physiology, and molecular regulation." Frontiers in Plant Science **13**: 994785.

Scott, N., et al. (1993). "Reactions of arsenic (III) and arsenic (V) species with glutathione." Chemical research in toxicology **6**(1): 102-106.

Sobariu, D. L., et al. (2017). "Rhizobacteria and plant symbiosis in heavy metal uptake and its implications for soil bioremediation." New biotechnology **39**: 125-134.

Tang, J., et al. (2019). "Diagnosis of soil contamination using microbiological indices: A review on heavy metal pollution." Journal of environmental management **242**: 121-130.

Trompeta, A.-F. A., et al. (2019). "Toxicity testing of MWCNTs to aquatic organisms." RSC Advances **9**(63): 36707-36716.

Vardhan, K. H., et al. (2019). "A review on heavy metal pollution, toxicity and remedial measures: Current trends and future perspectives." Journal of Molecular Liquids **290**: 111197.

Wang, J., et al. (2017). "Preparation of molybdenum disulfide coated Mg/Al layered double hydroxide composites for efficient removal of chromium (VI)." ACS Sustainable Chemistry & Engineering **5**(8): 7165-7174.

Wenzel, T. J. (2013). Douglas A. Skoog, Donald M. West, F. James Holler, and Stanley R. Crouch: Fundamentals of analytical chemistry, international ed, Springer.

Witkowska, D., et al. (2021). "Heavy metals and human health: Possible exposure pathways and the competition for protein binding sites." Molecules **26**(19): 6060.

Zhang, M., et al. (2019). "The spatial and seasonal variations of bacterial community structure and influencing factors in river sediments." Journal of environmental management **248**: 109293.

Zhou, S., et al. (2014). "Enhanced Cr (VI) removal from aqueous solutions using Ni/Fe bimetallic nanoparticles: characterization, kinetics and mechanism." RSC Advances **4**(92): 50699-50707.

Abbas, G., Murtaza, B., Bibi, I., Shahid, M., Niazi, N. K., Khan, M. I., . . . Natasha. (2018). Arsenic uptake, toxicity, detoxification, and speciation in plants: physiological, biochemical, and molecular aspects. *International journal of environmental research and public health, 15*(1), 59.

Abdulmalik, A. F., Yakasai, H. M., Usman, S., Muhammad, J. B., Jagaba, A. H., Ibrahim, S., . . . Shukor, M. Y. (2023). Characterization and invitro toxicity assay of bio-reduced hexavalent chromium by Acinetobacter sp. isolated from tannery effluent. *Case Studies in Chemical and Environmental Engineering, 8*, 100459.

Ahmad, T., Ahmad, K., Khan, Z. I., Munir, Z., Khalofah, A., Al-Qthanin, R. N., . . . Farooq, S. (2021). Chromium accumulation in soil, water and forage samples in automobile emission area. *Saudi Journal of Biological Sciences, 28*(6), 3517-3522.

Ahmed, M. F., Mokhtar, M. B., & Alam, L. (2021). Carcinogenic and non-carcinogenic health risk of arsenic ingestion via drinking water in Langat River Basin, Malaysia. *Environmental geochemistry and health, 43*, 897-914.

Ahmed, S. F., Kumar, P. S., Rozbu, M. R., Chowdhury, A. T., Nuzhat, S., Rafa, N., . . . Mofijur, M. (2022). Heavy metal toxicity, sources, and remediation techniques for contaminated water and soil. *Environmental Technology & Innovation, 25*, 102114.

Aigberua, A. O., & Izah, S. C. (2019). Macro nutrient and selected heavy metals in powered herbal medicine sold in Nigeria. *Int J Med Plants Nat Prod, 5*(1), 23-29.

Alessandrello, M., & Vullo, D. L. (2018). Biotreatment of Cr (VI)-containing wastewater mediated by indigenous bacteria.

Allevato, E., Stazi, S. R., Marabottini, R., & D'Annibale, A. (2019). Mechanisms of arsenic assimilation by plants and countermeasures to attenuate its accumulation in crops other than rice. *Ecotoxicology and environmental safety, 185*, 109701.

Alonso-Bernáldez, M., Cuevas-Sierra, A., Micó, V., Higuera-Gómez, A., Ramos-Lopez, O., Daimiel, L., . . . Ramirez de Molina, A. (2023). An Interplay between Oxidative Stress (Lactate Dehydrogenase) and Inflammation (Anisocytosis) Mediates COVID-19 Severity Defined by Routine Clinical Markers. *Antioxidants, 12*(2), 234.

Alseroury, F., Almeelbi, T., Khan, A., Barakata, M., Al-Zahrani, J., & Alali, W. (2018). Estimation of natural radioactive and heavy metals concentration in underground water. *Journal of radiation research and applied sciences, 11*(4), 373-378.

Aoshima, K. (2012). Itai-itai disease: cadmium-induced renal tubular osteomalacia. *Nihon eiseigaku zasshi. Japanese journal of hygiene, 67*(4), 455-463.

Ashour, E. A., & Tony, M. A. (2020). Eco-friendly removal of hexavalent chromium from aqueous solution using natural clay mineral: activation and modification effects. *SN Applied Sciences, 2*, 1-13.

ATSDR, U. (2012). Toxicological profile for chromium. *US Department of Health and Human Services, Public Health Service*.

Ballav, N., Maity, A., & Mishra, S. B. (2012). High efficient removal of chromium (VI) using glycine doped polypyrrole adsorbent from aqueous solution. *Chemical Engineering Journal, 198*, 536-546.

Banerjee, A., Tabassum, S. K., Debnath, D., Hazra, D., & Pal, R. (2022). A review on detoxification and bioremediation of antimony by bacterial species. *Acta Scientific MICROBIOLOGY (ISSN: 2581-3226), 5*(9).

Bhakta, J. N. (2017). Metal toxicity in microorganism *Handbook of research on inventive bioremediation techniques* (pp. 1-23): IGI Global.

Bhardwaj, A. (2022). Understanding the diversified microbial operon framework coupled to arsenic transformation and expulsion. *Biologia, 77*(12), 3531-3544.

Binkowski, Ł. J. (2019). Arsenic, As. *Mammals and Birds as Bioindicators of Trace Element Contaminations in Terrestrial Environments: An Ecotoxicological Assessment of the Northern Hemisphere*, 463-481.

Bora, F. D., Bunea, C. I., Chira, R., & Bunea, A. (2020). Assessment of the quality of polluted areas in northwest Romania based on the content of elements in different organs of grapevine (Vitis vinifera L.). *Molecules, 25*(3), 750.

Briffa, J., Sinagra, E., & Blundell, R. (2020). Heavy metal pollution in the environment and their toxicological effects on humans. *Heliyon, 6*(9).

Brito, E. M., De la Cruz Barrón, M., Caretta, C. A., Goñi-Urriza, M., Andrade, L. H., Cuevas-Rodríguez, G., . . . Guyoneaud, R. (2015). Impact of hydrocarbons, PCBs and heavy metals on bacterial communities in Lerma River, Salamanca, Mexico: investigation of hydrocarbon degradation potential. *Science of the total environment, 521*, 1-10.

Budnik, L. T., & Casteleyn, L. (2019). Mercury pollution in modern times and its socio-medical consequences. *Science of the total environment, 654*, 720-734.

Buendía-González, L., Orozco-Villafuerte, J., Cruz-Sosa, F., Barrera-Díaz, C., & Vernon-Carter, E. (2010). Prosopis laevigata a potential chromium (VI) and cadmium (II) hyperaccumulator desert plant. *Bioresource Technology, 101*(15), 5862-5867.

Carolin, C. F., Kumar, P. S., Saravanan, A., Joshiba, G. J., & Naushad, M. (2017). Efficient techniques for the removal of toxic heavy metals from aquatic environment: A review. *Journal of Environmental Chemical Engineering, 5*(3), 2782-2799.

Çelebi, H., Gök, G., & Gök, O. (2020). Adsorption capability of brewed tea waste in waters containing toxic lead (II), cadmium (II), nickel (II), and zinc (II) heavy metal ions. *Scientific reports, 10*(1), 17570.

Chen, J. P., & Yang, L. (2006). Study of a heavy metal biosorption onto raw and chemically modified Sargassum sp. via spectroscopic and modeling analysis. *Langmuir, 22*(21), 8906-8914.

Cherian, T., & Narayana, B. (2005). A new spectrophotometric method for the determination of arsenic in environmental and biological samples. *Analytical letters, 38*(13), 2207-2216.

Chibuike, G. U., & Obiora, S. C. (2014). Heavy metal polluted soils: effect on plants and bioremediation methods. *Applied and environmental soil science, 2014*.

Choppala, G., Bolan, N., & Park, J. H. (2013). Chromium contamination and its risk management in complex environmental settings. *Advances in agronomy, 120*, 129-172.

Chowdhury, U. (2022). Groundwater arsenic contamination status and its impact on humans' health in Bangladesh. *Academic Research Journal of Nature and Public Health, 1*(1), 1-12.

Chrysochoou, M., Theologou, E., Bompoti, N., Dermatas, D., & Panagiotakis, I. (2016). Occurrence, origin and transformation processes of geogenic chromium in soils and sediments. *Current Pollution Reports, 2*, 224-235.

Danouche, M., El Ghachtouli, N., & El Arroussi, H. (2021). Phycoremediation mechanisms of heavy metals using living green microalgae: physicochemical and molecular approaches for enhancing selectivity and removal capacity. *Heliyon, 7*(7).

Danouche, M., El Ghachtouli, N., El Baouchi, A., & El Arroussi, H. (2020). Heavy metals phycoremediation using tolerant green microalgae: Enzymatic and non-enzymatic antioxidant syplumules for the management of oxidative stress. *Journal of Environmental Chemical Engineering, 8*(5), 104460.

de Moura Sousa, L., Moreira, F. S., Cardoso, V. L., & Batista, F. R. X. (2023). Light intensity effect on the performance of Rhodobacter capsulatus in removal of chromium from effluent. *Journal of Water Process Engineering, 52*, 103567.

DesMarias, T. L., & Costa, M. (2019). Mechanisms of chromium-induced toxicity. *Current opinion in toxicology, 14*, 1-7.

Diaconu, M., Pavel, L. V., Hlihor, R.-M., Rosca, M., Fertu, D. I., Lenz, M., . . . Gavrilescu, M. (2020). Characterization of heavy metal toxicity in some plants and microorganisms—A preliminary approach for environmental bioremediation. *New biotechnology, 56*, 130-139.

Dodson, M., de la Vega, M. R., Harder, B., Castro-Portuguez, R., Rodrigues, S. D., Wong, P. K., . . . Zhang, D. D. (2018). Low-level arsenic causes proteotoxic stress and not oxidative stress. *Toxicology and applied pharmacology, 341*, 106-113.

Engwa, G. A., Ferdinand, P. U., Nwalo, F. N., & Unachukwu, M. N. (2019). Mechanism and health effects of heavy metal toxicity in humans. *Poisoning in the modern world-new tricks for an old dog, 10*, 70-90.

FANI, P. (2023). 12.1 Classification of Heavy Metals. *Nanoparticles as Sustainable Environmental Remediation Agents, 61*, 322.

Fashola, M., Anagun, O., & Babalola, O. O. (2023). Heavy metal pollution: Toxic effects on bacterial cells.

Felix, A., Gable, J., Vitale, R., Gratson, D., & Carriker, N. (2020). Challenges Associated with Generating Reliable Nanogram-per-Liter Measurements of Hexavalent Chromium in Groundwater. *Environmental Toxicology and Chemistry, 39*(8), 1526-1533.

Fernández, P. M., Viñarta, S. C., Bernal, A. R., Cruz, E. L., & Figueroa, L. I. (2018). Bioremediation strategies for chromium removal: current research, scale-up approach and future perspectives. *Chemosphere, 208*, 139-148.

Fu, Z., & Xi, S. (2020). The effects of heavy metals on human metabolism. *Toxicology mechanisms and methods, 30*(3), 167-176.

Gajbhiye, S., & Hirota, R. (1990). Toxicity of heavy metals to brine shrimp Artemia.

Ganie, S. Y., Javaid, D., Hajam, Y. A., & Reshi, M. S. (2024). Arsenic toxicity: sources, pathophysiology and mechanism. *Toxicology Research, 13*(1), tfad111.

Gautam, A., Kushwaha, A., & Rani, R. (2021). Microbial remediation of hexavalent chromium: An eco-friendly strategy for the remediation of chromium-contaminated wastewater *The future of effluent treatment plants* (pp. 361-384): Elsevier.

Gautam, P. K., Gautam, R. K., Banerjee, S., Chattopadhyaya, M., & Pandey, J. (2016). Heavy metals in the environment: fate, transport, toxicity and remediation technologies. *Nova Sci Publishers, 60*, 101-130.

Genchi, G., Lauria, G., Catalano, A., Carocci, A., & Sinicropi, M. S. (2021). The double face of metals: The intriguing case of chromium. *Applied Sciences, 11*(2), 638.

Goncharuk, E. A., & Zagoskina, N. V. (2023). Heavy metals, their phytotoxicity, and the role of phenolic antioxidants in plant stress responses with focus on cadmium. *Molecules, 28*(9), 3921.

GracePavithra, K., Jaikumar, V., Kumar, P. S., & SundarRajan, P. (2019). A review on cleaner strategies for chromium industrial wastewater: present research and future perspective. *Journal of Cleaner Production, 228*, 580-593.

Grath, M. (2017). Alarmingly high'levels of arsenic in Pakistan's ground water.

Gregory, P. J. (2022). RUSSELL REVIEW Are plant radicles only "in" soil or are they "of" it? Radicles, soil formation and function. *European Journal of Soil Science, 73*(1), e13219.

Gu, H., Liu, X., Wang, S., Chen, Z., Yang, H., Hu, B., . . . Wang, X. (2022). COF-based composites: extraordinary removal performance for heavy metals and radionuclides from aqueous solutions. *Reviews of Environmental Contamination and Toxicology, 260*(1), 23.

Guha, G., Rajkumar, V., Kumar, R. A., & Mathew, L. (2011). Antioxidant activity of Lawsonia inermis extracts inhibits chromium (VI)-induced cellular and DNA toxicity. *Evidence-Based Complementary and Alternative Medicine, 2011*.

Guo, S., Xiao, C., Zhou, N., & Chi, R. (2021). Speciation, toxicity, microbial remediation and phytoremediation of soil chromium contamination. *Environmental Chemistry Letters, 19*, 1413-1431.

Gupta, A., Dubey, P., Kumar, M., Roy, A., Sharma, D., Khan, M. M., . . . Hasanuzzaman, M. (2022). Consequences of arsenic contamination on plants and mycoremediation-mediated arsenic stress tolerance for sustainable agriculture. *Plants, 11*(23), 3220.

Gurnari, C., De Bellis, E., Divona, M., Ottone, T., Lavorgna, S., & Voso, M. T. (2020). When poisons cure: the case of arsenic in acute promyelocytic leukemia. *Chemotherapy, 64*(5-6), 238-247.

Hassan, M. M. (2018). *Arsenic in groundwater: poisoning and risk assessment*: Crc Press.

Hausladen, D. M., Alexander-Ozinskas, A., McClain, C., & Fendorf, S. (2018). Hexavalent chromium sources and distribution in California groundwater. *Environmental science & technology, 52*(15), 8242-8251.

Haynes, W. M. (2014). *CRC handbook of chemistry and physics*: CRC press.

Hlihor, R. M., Bulgariu, L., Sobariu, D. L., Diaconu, M., Tavares, T., & Gavrilescu, M. (2014). Recent advances in biosorption of heavy metals: support tools for biosorption equilibrium, kinetics and mechanism.

HUVINEN, M. Exposure to Chromium and its Long-term.

Jacobs, J. A., & Avakian, C. P. (2005). *Chromium (VI) handbook*: Taylor & Francis.

Ji, L., Song, P., Zhou, Y., Sun, X.-L., Gao, Y., & Tang, Y. (2023). A tridentate phenoxy-phosphine (POP) divalent chromium complex and its reactivities in olefin polymerization. *Polymer Chemistry, 14*(6), 763-772.

Jiménez, J.-R., Doistau, B., Poncet, M., & Piguet, C. (2021). Heteroleptic trivalent chromium in coordination chemistry: Novel building blocks for addressing old challenges in multimetallic luminescent complexes. *Coordination Chemistry Reviews, 434*, 213750.

Jobby, R., Jha, P., Gupta, A., Gupte, A., & Desai, N. (2019). Bioremoval of chromium by radicle nodule bacteria Sinorhizobium sp. SAR1. *PloS one, 14*(7), e0219387.

Jobby, R., Jha, P., Yadav, A. K., & Desai, N. (2018). Biosorption and bioremoval of hexavalent chromium [Cr (VI)]: a comprehensive review. *Chemosphere, 207*, 255-266.

Joshi, S., Gangola, S., Bhandari, G., Bhandari, N. S., Nainwal, D., Rani, A., . . . Slama, P. (2023). Rhizospheric bacteria: the key to sustainable heavy metal detoxification strategies. *Frontiers in microbiology, 14*.

Jutsz, A. M., & Gnida, A. (2015). Mechanisms of stress avoidance and tolerance by plants used in phytoremediation of heavy metals. *Archives of Environmental Protection, 41*(4), 104.

Kabiraj, A., Biswas, R., Halder, U., & Bandopadhyay, R. (2022). Bacterial arsenic metabolism and its role in arsenic bioremediation. *Current Microbiology, 79*(5), 131.

Kandhol, N., Aggarwal, B., Bansal, R., Parveen, N., Singh, V. P., Chauhan, D. K., . . . Peralta-Videa, J. (2022). Nanoparticles as a potential protective agent for arsenic toxicity alleviation in plants. *Environmental Pollution, 300*, 118887.

Karthik, C., Ramkumar, V. S., Pugazhendhi, A., Gopalakrishnan, K., & Arulselvi, P. I. (2017). Biosorption and bioremoval of Cr (VI) by novel Cellulosimicrobium funkei strain AR6. *Journal of the Taiwan Institute of Chemical Engineers, 70*, 282-290.

Khalef, R. N., Hassan, A. I., & Saleh, H. M. (2022). Heavy metal's environmental impact.

Khanna, K., Jamwal, V. L., Gandhi, S. G., Ohri, P., & Bhardwaj, R. (2019). Metal resistant PGPR lowered Cd uptake and expression of metal transporter genes with improved growth and photosynthetic pigments in Lycopersicon esculentum under metal toxicity. *Scientific reports, 9*(1), 5855.

Kile, M. L., Hoffman, E., Rodrigues, E. G., Breton, C. V., Quamruzzaman, Q., Rahman, M., . . . Christiani, D. C. (2011). A pathway-based analysis of urinary arsenic metabolites and skin lesions. *American journal of epidemiology, 173*(7), 778-786.

Kim, H. S., Kim, Y. J., & Seo, Y. R. (2015). An overview of carcinogenic heavy metal: molecular toxicity mechanism and prevention. *Journal of cancer prevention, 20*(4), 232.

Kim, N. H., Hyun, Y. Y., Lee, K.-B., Chang, Y., Rhu, S., Oh, K.-H., & Ahn, C. (2015). Environmental heavy metal exposure and chronic kidney disease in the general population. *Journal of Korean medical science, 30*(3), 272-277.

Kuehr, S., Kosfeld, V., & Schlechtriem, C. (2021). Bioaccumulation assessment of nanomaterials using freshwater invertebrate species. *Environmental Sciences Europe, 33*, 1-36.

Kumar, A., Hussain, T., Susmita, C., Maurya, D. K., Danish, M., & Farooqui, S. A. (2021). Microbial remediation and detoxification of heavy metals by plants and microbes *The future of effluent treatment plants* (pp. 589-614): Elsevier.

Kumar, V., Swain, H. S., Upadhyay, A., Ramteke, M. H., Sarkar, D. J., Roy, S., & Das, B. K. (2023). Bioaccumulation of Potentially Toxic Elements in Commercially Important Food Fish Species from Lower Gangetic Stretch: Food Security and Human Health Risk Assessment. *Biological Trace Element Research*, 1-14.

Lace, A., Ryan, D., Bowkett, M., & Cleary, J. (2019). Chromium monitoring in water by colorimetry using optimised 1, 5-diphenylcarbazide method. *International journal of environmental research and public health, 16*(10), 1803.

Lepp, N., & Dickinson, N. (1994). Fungicide-derived copper in tropical plantation crops. *Toxic metals in soil-plant syplumules.*, 367-393.

Liu, G.-h., Tang, X., Yuan, J., Li, Q., Qi, L., Wang, H., . . . Zhao, Q. (2023). Activated sludge process enabling highly efficient removal of heavy metal in wastewater. *Environmental Science and Pollution Research, 30*(8), 21132-21143.

Liu, W., Mei, Y., Etschmann, B., Brugger, J., Pearce, M., Ryan, C. G., . . . Paterson, D. (2017). Arsenic in hydrothermal apatite: Oxidation state, mechanism of uptake, and comparison between experiments and nature. *Geochimica et Cosmochimica Acta, 196*, 144-159.

Liu, X., Zhang, S., Shan, X., & Zhu, Y.-G. (2005). Toxicity of arsenate and arsenite on germination, seedling growth and amylolytic activity of wheat. *Chemosphere, 61*(2), 293-301.

Machado-Neves, M., & Souza, A. C. F. (2023). The effect of arsenical compounds on mitochondrial metabolism *Mitochondrial intoxication* (pp. 379-407): Elsevier.

Maciag, B., Brenan, J., & Keltie, E. (2018). Valence state of arsenic and antimony in basalts: an experimental and synchrotron study. *Atlantic Geology, 54*.

Manu, M., Onete, M., & Băncilă, R. I. (2018). The effect of heavy metals on mite communities (Acari: Gamasina) from urban parks-Bucharest, Romania. *Environmental Engineering & Management Journal (EEMJ), 17*(9).

Masindi, V., & Muedi, K. L. (2018). Environmental contamination by heavy metals. *Heavy metals, 10*, 115-132.

Medda, N., Patra, R., Ghosh, T. K., & Maiti, S. (2020). Neurotoxic mechanism of arsenic: synergistic effect of mitochondrial instability, oxidative stress, and hormonal-neurotransmitter impairment. *Biological Trace Element Research, 198*, 8-15.

Moiseenko, T., & Gashkina, N. (2020). Distribution and bioaccumulation of heavy metals (Hg, Cd and Pb) in fish: Influence of the aquatic environment and climate. *Environmental Research Letters, 15*(11), 115013.

Mondal, B., Maulik, D., Mandal, M., Sarkar, G. N., Sengupta, S., & Ghosh, D. (2017). Analysis of carcinogenic heavy metals in gallstones and its role in gallbladder carcinogenesis. *Journal of Gastrointestinal Cancer, 48*, 361-368.

Monga, A., Fulke, A. B., & Dasgupta, D. (2022). Recent developments in essentiality of trivalent chromium and toxicity of hexavalent chromium: implications on human health and remediation strategies. *Journal of Hazardous Materials Advances, 7*, 100113.

Muzaffar, S., Khan, J., Srivastava, R., Gorbatyuk, M. S., & Athar, M. (2023). Mechanistic understanding of the toxic effects of arsenic and warfare arsenicals on human health and environment. *Cell Biology and Toxicology, 39*(1), 85-110.

Natasha, Bibi, I., Hussain, K., Amen, R., Hasan, I. M. U., Shahid, M., . . . Asghar, H. N. (2021). The potential of microbes and sulfate in reducing arsenic phytoaccumulation by maize (Zea mays L.) plants. *Environmental geochemistry and health*, 1-15.

Nigam, S., Gopal, K., & Vankar, P. S. (2013). Biosorption of arsenic in drinking water by submerged plant: Hydrilla verticilata. *Environmental Science and Pollution Research, 20*, 4000-4008.

Nurzhan, A., Tian, H., Nuralykyzy, B., & He, W. (2022). Soil enzyme activities and enzyme activity indices in long-term arsenic-contaminated soils. *Eurasian Soil Science, 55*(10), 1425-1435.

Nwokocha, C. R., Owu, D. U., Nwokocha, M. I., Ufearo, C. S., & Iwuala, M. O. (2012). Comparative study on the efficacy of Allium sativum (garlic) in reducing some heavy metal accumulation in liver of wistar rats. *Food and chemical toxicology, 50*(2), 222-226.

Orman, S., Ok, H., & Kaplan, M. (2014). Application of Sewage Sludge for Growing Alfalfa, Its Effects on the Macro-Micronutrient Concentration, Heavy Metal Accumulation, and Translocation. *Ekoloji Dergisi, 23*(90).

Oves, M., Khan, M. S., & Zaidi, A. (2013). Biosorption of heavy metals by Bacillus thuringiensis strain OSM29 originating from industrial effluent contaminated north Indian soil. *Saudi Journal of Biological Sciences, 20*(2), 121-129.

Ozturk, M., Metin, M., Altay, V., Bhat, R. A., Ejaz, M., Gul, A., . . . Nahar, K. (2022). Arsenic and human health: genotoxicity, epigenomic effects, and cancer signaling. *Biological Trace Element Research*, 1-14.

Paithankar, J. G., Saini, S., Dwivedi, S., Sharma, A., & Chowdhuri, D. K. (2021). Heavy metal associated health hazards: An interplay of oxidative stress and signal transduction. *Chemosphere, 262*, 128350.

Pal, S., Sarkar, K., Nath, P. P., Mondal, M., Khatun, A., & Paul, G. (2017). Bisphenol S impairs blood functions and induces cardiovascular risks in rats. *Toxicology reports, 4*, 560-565.

Patlolla, A. K., Barnes, C., Yedjou, C., Velma, V., & Tchounwou, P. B. (2009). Oxidative stress, DNA damage, and antioxidant enzyme activity induced by hexavalent chromium in Sprague-Dawley rats. *Environmental Toxicology: An International Journal, 24*(1), 66-73.

Paula, J. F., Froes-Silva, R. E., & Ciminelli, V. S. (2012). Arsenic determination in complex mining residues by ICP OES after ultrasonic extraction. *Microchemical Journal, 104*, 12-16.

Pavel, V. L., Sobariu, D.-L., Fertu, I. D. T., Statescu, F., & Gavrilescu, M. (2013). Symbiosis in the environment biomanagement of soils contaminated with heavy metals. *Eur. J. Sci. Theol, 9*(4), 211.

Pavel, V. L., Sobariu, D. L., Diaconu, M., Stătescu, F., & Gavrilescu, M. (2013). Effects of heavy metals on Lepidium sativum germination and growth. *Environmental Engineering & Management Journal (EEMJ), 12*(4).

Peralta, J., Gardea-Torresdey, J. L., Tiemann, K., Gomez, E., Arteaga, S., Rascon, E., & Parsons, J. (2001). Uptake and effects of five heavy metals on seed germination and plant growth in alfalfa (Medicago sativa L.). *Bulletin of Environmental Contamination and toxicology, 66*, 727-734.

Prakash, S., & Verma, A. K. (2021). Arsenic: It's toxicity and impact on human health. *International Journal of Biological Innovations, IJBI, 3*(1), 38-47.

Pujari, M., & Kapoor, D. (2021). Heavy metals in the ecosyplumule: Sources and their effects *Heavy metals in the environment* (pp. 1-7): Elsevier.

Qian, H., Li, Q., Sun, J., Xaikoua, S., & Sun, H. (2020). Characterization and Analysis of Chromium Coating Electrodeposited on Brass in ChCl-EG Deep Eutectic Solvent. *International Journal of Electrochemical Science, 15*(9), 8960-8970.

Rahman, M. M., Chowdhury, U. K., Mukherjee, S. C., Mondal, B. K., Paul, K., Lodh, D., . . . Saha, K. C. (2001). Chronic arsenic toxicity in Bangladesh and West Bengal, India—a review and commentary. *Journal of Toxicology: Clinical Toxicology, 39*(7), 683-700.

Rahman, Z., & Thomas, L. (2021). Chemical-assisted microbially mediated chromium (Cr)(VI) reduction under the influence of various electron donors, redox mediators, and other additives: an outlook on enhanced Cr (VI) removal. *Frontiers in microbiology, 11*, 619766.

Raju, C. V., Cho, C. H., Rani, G. M., Manju, V., Umapathi, R., Huh, Y. S., & Park, J. P. (2023). Emerging insights into the use of carbon-based nanomaterials for the electrochemical detection of heavy metal ions. *Coordination Chemistry Reviews, 476*, 214920.

Raju, N. J. (2022). Arsenic in the geo-environment: A review of sources, geochemical processes, toxicity and removal technologies. *Environmental research, 203*, 111782.

Ramli, N. N., Othman, A. R., Kurniawan, S. B., Abdullah, S. R. S., & Hasan, H. A. (2023). Metabolic pathway of Cr (VI) reduction by bacteria: a review. *Microbiological research, 268*, 127288.

Recio-Vega, R., Hernandez-Gonzalez, S., Michel-Ramirez, G., Olivas-Calderón, E., Lantz, R. C., Gandolfi, A. J., & Amistadi, M. K. (2021). Association between the polymorphism of three genes involved in the methylation and efflux of arsenic (As3MT, MRP1, and P-gp) with lung cancer in a Mexican cohort. *Journal of Applied Toxicology, 41*(9), 1357-1366.

Rehman, K., Fatima, F., Waheed, I., & Akash, M. S. H. (2018). Prevalence of exposure of heavy metals and their impact on health consequences. *Journal of cellular biochemistry, 119*(1), 157-184.

Renu, K., Chakraborty, R., Myakala, H., Koti, R., Famurewa, A. C., Madhyastha, H., . . . Gopalakrishnan, A. V. (2021). Molecular mechanism of heavy metals (Lead, Chromium, Arsenic, Mercury, Nickel and Cadmium)-induced hepatotoxicity–A review. *Chemosphere, 271*, 129735.

Sahoo, K., & Sharma, A. (2023). Understanding the mechanistic roles of environmental heavy metal stressors in regulating ferroptosis: Adding new paradigms to the links with diseases. *Apoptosis, 28*(3-4), 277-292.

Sarkar, A., & Paul, B. (2016). The global menace of arsenic and its conventional remediation-A critical review. *Chemosphere, 158*, 37-49.

Saud, S., Wang, D., Fahad, S., Javed, T., Jaremko, M., Abdelsalam, N. R., & Ghareeb, R. Y. (2022). The impact of chromium ion stress on plant growth, developmental physiology, and molecular regulation. *Frontiers in Plant Science, 13*, 994785.

Scott, N., Hatlelid, K. M., MacKenzie, N. E., & Carter, D. E. (1993). Reactions of arsenic (III) and arsenic (V) species with glutathione. *Chemical research in toxicology, 6*(1), 102-106.

Shadreck, M., & Mugadza, T. (2013). Chromium, an essential nutrient and pollutant: A review. *African Journal of Pure and Applied Chemistry, 7*(9), 310-317.

Shah, S. B. (2021). Heavy metals in the marine environment—an overview. *Heavy Metals in Scleractinian Corals*, 1-26.

Shaji, E., Santosh, M., Sarath, K., Prakash, P., Deepchand, V., & Divya, B. (2021). Arsenic contamination of groundwater: A global synopsis with focus on the Indian Peninsula. *Geoscience frontiers, 12*(3), 101079.

Shanker, A. K., Cervantes, C., Loza-Tavera, H., & Avudainayagam, S. (2005). Chromium toxicity in plants. *Environment international, 31*(5), 739-753.

Sharma, P., & Singh, G. (2016). Mercury toxicity and its management: need of the hour. *The Pharma Innovation, 5*(10, Part B), 96.

Sharma, P., Singh, S. P., Parakh, S. K., & Tong, Y. W. (2022). Health hazards of hexavalent chromium (Cr (VI)) and its microbial reduction. *Bioengineered, 13*(3), 4923-4938.

Sharma, P., Tripathi, S., Vadakedath, N., & Chandra, R. (2021). In-situ toxicity assessment of pulp and paper industry wastewater on Trigonella foenum-graecum L: Potential source of cytotoxicity and chromosomal damage. *Environmental Technology & Innovation, 21*, 101251.

Sharma, V. K., Shah, M. P., Parmar, S., & Kumar, A. (2020). *Fungi Bio-prospects in Sustainable Agriculture, Environment and Nano-technology: Volume 1: Fungal Diversity of Sustainable Agriculture*: Academic Press.

Sheeja, christobel, & Lipton, A. (2016). International Journal of Application or Innovation in Engineering & Management (IJAIEM). *International Journal of Application or Innovation in Engineering & Management (IJAIEM), 5*, 100-105.

Siddiqui, S. I., Singh, P. N., Tara, N., Pal, S., Chaudhry, S. A., & Sinha, I. (2020). Arsenic removal from water by starch functionalized maghemite nano-adsorbents: Thermodynamics and kinetics investigations. *Colloid and Interface Science Communications, 36*, 100263.

Siedlecka, A. (1995). Some aspects of interactions between heavy metals and plant mineral nutrients. *Acta Societatis Botanicorum Poloniae, 64*(3), 265-272.

Silver, S. (1996). Bacterial resistances to toxic metal ions-a review. *Gene, 179*(1), 9-19.

Singh, A., Porwal, S., & Varma, A. (2021). Hexavalent Chromium: toxic and genotoxic effects and its bioremediation strategies. *Biomed J Sci Tech Res, 35*(3), 27637-27643.

Singh, B. R., & Steinnes, E. (2020). Soil and water contamination by heavy metals *Soil processes and water quality* (pp. 233-271): CRC Press.

Singh, D. P., Yadav, S. K., Patel, K., Patel, S., Patil, G. P., Bijalwan, V., . . . Boparai, R. K. (2023). Short-term trivalent arsenic and hexavalent chromium exposures induce gut dysbiosis and transcriptional alteration in adipose tissue of mice. *Molecular Biology Reports, 50*(2), 1033-1044.

Singh, H. P., Mahajan, P., Kaur, S., Batish, D. R., & Kohli, R. K. (2013). Chromium toxicity and tolerance in plants. *Environmental Chemistry Letters, 11*, 229-254.

Singh, S., Kang, S. H., Mulchandani, A., & Chen, W. (2008). Bioremediation: environmental clean-up through pathway engineering. *Current opinion in biotechnology, 19*(5), 437-444.

Sisodia, A. S. (2023). Heavy Metals and Zinc Levels Roles in the Environment and their use on Higher Plants. *Applied Science and Biotechnology Journal for Advanced Research, 2*(5), 23-27.

Sobariu, D. L., Fertu, D. I. T., Diaconu, M., Pavel, L. V., Hlihor, R.-M., Drăgoi, E. N., . . . Gavrilescu, M. (2017). Rhizobacteria and plant symbiosis in heavy metal uptake and its implications for soil bioremediation. *New biotechnology, 39*, 125-134.

Srivastava, S. (2020). *Arsenic in drinking water and food*: Springer.

Srushti, S., Kumar, M., Hegde, S., Biradar, S., Shreedevi, S., Prashanth, S., & Babu, R. (2023). Role of Activator Protein-1 Transcription Factor in Oral Cancer. *Current Cancer Therapy Reviews, 19*(2), 103-116.

Stambulska, U. Y., Bayliak, M. M., & Lushchak, V. I. (2018). Chromium (VI) toxicity in legume plants: modulation effects of rhizobial symbiosis. *BioMed research international, 2018*.

Steinmetz-Späh, J. (2023). *Studies on Anti-Inflammatory and Vasoactive Effects of MPGES-1 Inhibition.* Karolinska Institutet (Sweden).

Su, X., Kushima, A., Halliday, C., Zhou, J., Li, J., & Hatton, T. A. (2018). Electrochemically-mediated selective capture of heavy metal chromium and arsenic oxyanions from water. *Nature Communications, 9*(1), 4701. doi:10.1038/s41467-018-07159-0

Tang, J., Zhang, J., Ren, L., Zhou, Y., Gao, J., Luo, L., . . . Chen, A. (2019). Diagnosis of soil contamination using microbiological indices: A review on heavy metal pollution. *Journal of environmental management, 242*, 121-130.

Tanu, F. Z., Hakim, A., & Hoque, S. (2016). Bacterial tolerance and reduction of chromium (VI) by Bacillus cereus isolate PGBw4. *American Journal of Environmental Protection, 5*(2), 35-38.

Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., & Sutton, D. J. (2012). Heavy metal toxicity and the environment. *Molecular, clinical and environmental toxicology: volume 3: environmental toxicology*, 133-164.

Tengku-Mazuki, T. A., Darham, S., Convey, P., Shaharuddin, N. A., Zulkharnain, A., Khalil, K. A., . . . Gomez-Fuentes, C. (2023). Effects of heavy metals on bacterial growth parameters in degradation of phenol by an Antarctic bacterial consortium. *Brazilian Journal of Microbiology*, 1-9.

Thomas, D. J., Li, J., Waters, S. B., Xing, W., Adair, B. M., Drobna, Z., . . . Styblo, M. (2007). Arsenic (+ 3 oxidation state) methyltransferase and the methylation of arsenicals. *Experimental Biology and Medicine, 232*(1), 3-13.

Trompeta, A.-F. A., Preiss, I., Ben-Ami, F., Benayahu, Y., & Charitidis, C. A. (2019). Toxicity testing of MWCNTs to aquatic organisms. *RSC Advances, 9*(63), 36707-36716.

Tunali, M., Tunali, M. M., & Yenigun, O. (2021). Characterization of different types of electronic waste: heavy metal, precious metal and rare earth element content by comparing different digestion methods. *Journal of Material Cycles and Waste Management, 23*, 149-157.

Ukhurebor, K. E., Aigbe, U. O., Onyancha, R. B., Nwankwo, W., Osibote, O. A., Paumo, H. K., . . . Siloko, I. U. (2021). Effect of hexavalent chromium on the environment and removal techniques: a review. *Journal of environmental management, 280*, 111809.

Ungurianu, A., Zanfirescu, A., Nițulescu, G., & Margină, D. (2021). Vitamin E beyond its antioxidant label. *Antioxidants, 10*(5), 634.

Van den Kerkhof, M., Leprohon, P., Mabille, D., Hendrickx, S., Tulloch, L. B., Wall, R. J., . . . Braillard, S. (2021). Identification of resistance determinants for a promising antileishmanial oxaborole series. *Microorganisms, 9*(7), 1408.

Vardhan, K. H., Kumar, P. S., & Panda, R. C. (2019). A review on heavy metal pollution, toxicity and remedial measures: Current trends and future perspectives. *Journal of Molecular Liquids, 290*, 111197.

Vishnoi, N., Dixit, S., & Singh, D. (2014). Surface binding and intracellular uptake of arsenic in bacteria isolated from arsenic contaminated site. *Ecological engineering, 73*, 569-578.

Walker, C. H., Sibly, R., Hopkin, S. P., & Peakall, D. B. (2012). *Principles of ecotoxicology*: CRC press.

Wallace, D. R., & Djordjevic, A. B. (2020). Heavy metal and pesticide exposure: A mixture of potential toxicity and carcinogenicity. *Current opinion in toxicology, 19*, 72-79.

Wang, J., Wang, P., Wang, H., Dong, J., Chen, W., Wang, X., . . . Wang, X. (2017). Preparation of molybdenum disulfide coated Mg/Al layered double hydroxide composites for efficient removal of chromium (VI). *ACS Sustainable Chemistry & Engineering, 5*(8), 7165-7174.

Wang, X., Li, Q., Liao, Q., Yan, Y., Xia, J., Lin, Q., . . . Liang, Y. (2020). Arsenic (III) bioremoval to tooeleite associated with the oxidation of Fe (II) via Acidithiobacillus ferrooxidans. *Chemosphere, 248*, 126080.

Wenzel, T. J. (2013). Douglas A. Skoog, Donald M. West, F. James Holler, and Stanley R. Crouch: Fundamentals of analytical chemistry, international ed: Springer.

Wise Jr, J. P., Young, J. L., Cai, J., & Cai, L. (2022). Current understanding of hexavalent chromium [Cr (VI)] neurotoxicity and new perspectives. *Environment international, 158*, 106877.

Witkowska, D., Słowik, J., & Chilicka, K. (2021). Heavy metals and human health: Possible exposure pathways and the competition for protein binding sites. *Molecules, 26*(19), 6060.

Wu, X., Cobbina, S. J., Mao, G., Xu, H., Zhang, Z., & Yang, L. (2016). A review of toxicity and mechanisms of individual and mixtures of heavy metals in the environment. *Environmental Science and Pollution Research, 23*, 8244-8259.

Yan, X., Wang, J., Zhu, L., Wang, J., Li, S., & Kim, Y. M. (2021). Oxidative stress, growth inhibition, and DNA damage in earthworms induced by the combined pollution of typical neonicotinoid insecticides and heavy metals. *Science of the total environment, 754*, 141873.

Yang, W., Song, W., Li, J., & Zhang, X. (2020). Bioleaching of heavy metals from wastewater sludge with the aim of land application. *Chemosphere, 249*, 126134.

Yang, X.-F., Liu, Y., Tao, W.-Q., Wang, S., Ren, P., Yang, S.-L., . . . Shi, W.-Q. (2022). Lipophilic phenanthroline diamide ligands in 1-octanol for separation of Am (III) from Eu (III). *Journal of Environmental Chemical Engineering, 10*(5), 108401.

Yu, X.-Z., Lin, Y.-J., & Zhang, Q. (2019). Metallothioneins enhance chromium detoxification through scavenging ROS and stimulating metal chelation in Oryza sativa. *Chemosphere, 220*, 300-313.

Yun, Y.-S., Park, D., Park, J. M., & Volesky, B. (2001). Biosorption of trivalent chromium on the brown seaweed biomass. *Environmental science & technology, 35*(21), 4353-4358.

Zakaria, Z. A., Zakaria, Z., Surif, S., & Ahmad, W. A. (2007). Hexavalent chromium reduction by Acinetobacter haemolyticus isolated from heavy-metal contaminated wastewater. *Journal of hazardous materials, 146*(1-2), 30-38.

Zeng, J., Ke, W., Deng, M., Tan, J., Li, C., Cheng, Y., & Xue, S. (2023). A practical method for identifying key factors in the distribution and formation of heavy metal pollution at a smelting site. *Journal of Environmental Sciences, 127*, 552-563.

Zhang, C., Lin, T., Nie, G., Hu, R., Pi, S., Wei, Z., . . . Hu, G. (2022). In vivo assessment of molybdenum and cadmium co-induce nephrotoxicity via causing calcium homeostasis disorder and autophagy in ducks (Anas platyrhyncha). *Ecotoxicology and environmental safety, 230*, 113099.

Zhang, J., Liu, J., Zheng, F., Yu, M., Shabala, S., & Song, W.-Y. (2022). Comparative analysis of arsenic transport and tolerance mechanisms: Evolution from prokaryote to higher plants. *Cells, 11*(17), 2741.

Zhang, M., Wu, Z., Sun, Q., Ding, Y., Ding, Z., & Sun, L. (2019). The spatial and seasonal variations of bacterial community structure and influencing factors in river sediments. *Journal of environmental management, 248*, 109293.

Zhou, J. R., Kaur, G., Ma, Y., Arutyunov, D., Lu, X., Le, X. C., & Leslie, E. M. (2021). Biliary excretion of arsenic by human HepaRG cells is stimulated by selenide and mediated by the multidrug resistance protein 2 (MRP2/ABCC2). *Biochemical Pharmacology, 193*, 114799.

Zhou, S., Li, Y., Chen, J., Liu, Z., Wang, Z., & Na, P. (2014). Enhanced Cr (VI) removal from aqueous solutions using Ni/Fe bimetallic nanoparticles: characterization, kinetics and mechanism. *RSC Advances, 4*(92), 50699-50707.

Alonso-Bernáldez, M., et al. (2023). "An Interplay between Oxidative Stress (Lactate Dehydrogenase) and Inflammation (Anisocytosis) Mediates COVID-19 Severity Defined by Routine Clinical Markers." Antioxidants **12**(2): 234.

Buendía-González, L., et al. (2010). "Prosopis laevigata a potential chromium (VI) and cadmium (II) hyperaccumulator desert plant." Bioresource Technology **101**(15): 5862-5867.

Çelebi, H., et al. (2020). "Adsorption capability of brewed tea waste in waters containing toxic lead (II), cadmium (II), nickel (II), and zinc (II) heavy metal ions." Scientific reports **10**(1): 17570.

de Moura Sousa, L., et al. (2023). "Light intensity effect on the performance of Rhodobacter capsulatus in removal of chromium from effluent." Journal of Water Process Engineering **52**: 103567.

DesMarias, T. L. and M. Costa (2019). "Mechanisms of chromium-induced toxicity." Current opinion in toxicology **14**: 1-7.

Diaconu, M., et al. (2020). "Characterization of heavy metal toxicity in some plants and microorganisms—A preliminary approach for environmental bioremediation." New biotechnology **56**: 130-139.

Gajbhiye, S. and R. Hirota (1990). "Toxicity of heavy metals to brine shrimp Artemia."

Kabiraj, A., et al. (2022). "Bacterial arsenic metabolism and its role in arsenic bioremediation." Current Microbiology **79**(5): 131.

Kumar, V., et al. (2023). "Bioaccumulation of Potentially Toxic Elements in Commercially Important Food Fish Species from Lower Gangetic Stretch: Food Security and Human Health Risk Assessment." Biological Trace Element Research: 1-14.

Muzaffar, S., et al. (2023). "Mechanistic understanding of the toxic effects of arsenic and warfare arsenicals on human health and environment." Cell Biology and Toxicology **39**(1): 85-110.

Pavel, V. L., et al. (2013). "Effects of heavy metals on Lepidium sativum germination and growth." Environmental Engineering & Management Journal (EEMJ) **12**(4).

Peralta, J., et al. (2001). "Uptake and effects of five heavy metals on seed germination and plant growth in alfalfa (Medicago sativa L.)." Bulletin of Environmental Contamination and toxicology **66**: 727-734.

Pujari, M. and D. Kapoor (2021). Heavy metals in the ecosyplumule: Sources and their effects. Heavy metals in the environment, Elsevier**:** 1-7.

Ramli, N. N., et al. (2023). "Metabolic pathway of Cr (VI) reduction by bacteria: a review." Microbiological research **268**: 127288.

Silver, S. (1996). "Bacterial resistances to toxic metal ions-a review." Gene **179**(1): 9-19.

Singh, D. P., et al. (2023). "Short-term trivalent arsenic and hexavalent chromium exposures induce gut dysbiosis and transcriptional alteration in adipose tissue of mice." Molecular Biology Reports **50**(2): 1033-1044.

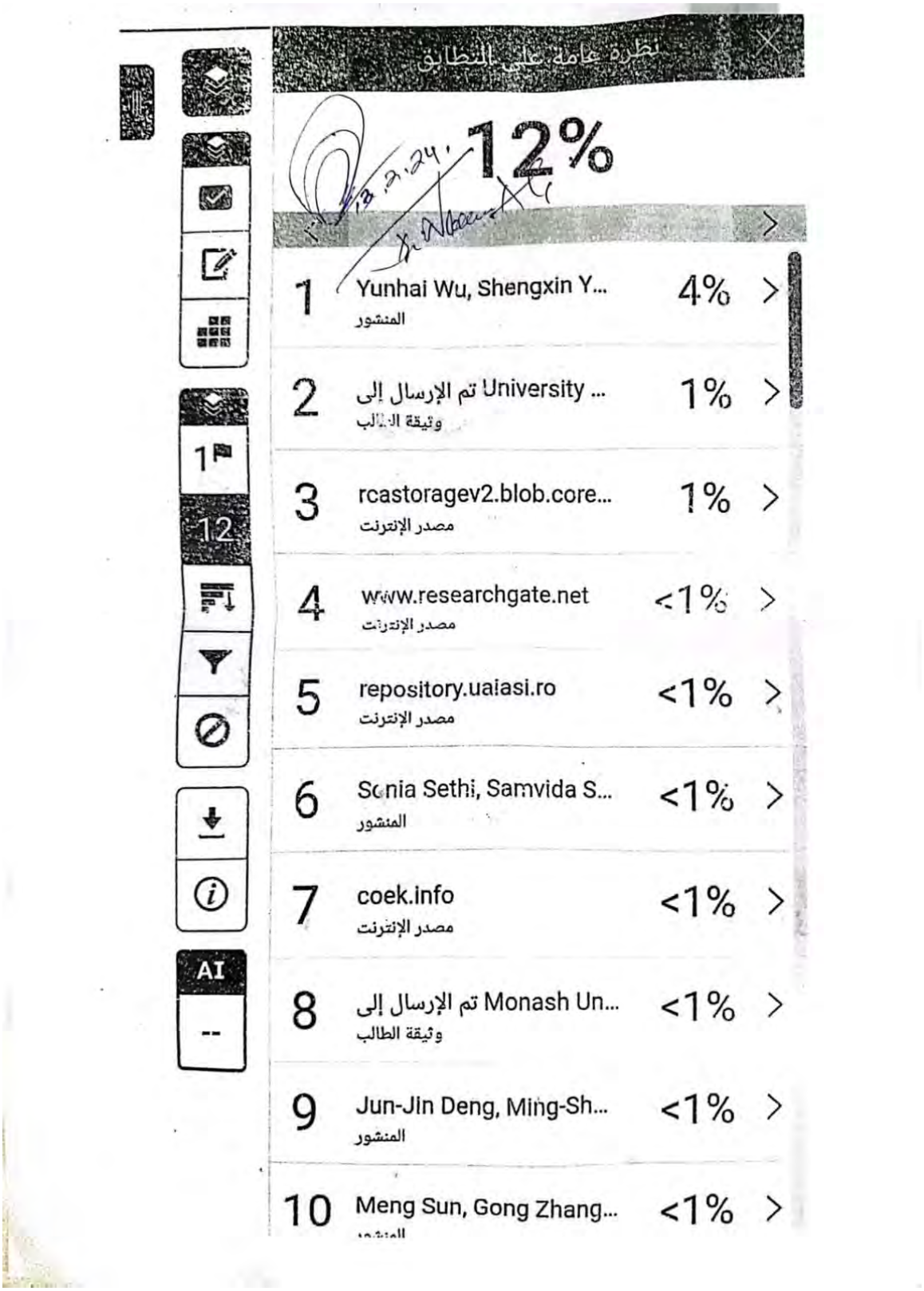
Singh, H. P., et al. (2013). "Chromium toxicity and tolerance in plants." Environmental Chemistry Letters **11**: 229-254.

Su, X., et al. (2018). "Electrochemically-mediated selective capture of heavy metal chromium and arsenic oxyanions from water." Nature Communications **9**(1): 4701.

Tanu, F. Z., et al. (2016). "Bacterial tolerance and reduction of chromium (VI) by Bacillus cereus isolate PGBw4." American Journal of Environmental Protection **5**(2): 35-38.

Tengku-Mazuki, T. A., et al. (2023). "Effects of heavy metals on bacterial growth parameters in degradation of phenol by an Antarctic bacterial consortium." Brazilian Journal of Microbiology: 1-9.

Wang, X., et al. (2020). "Arsenic (III) bioremoval to tooeleite associated with the oxidation of Fe (II) via Acidithiobacillus ferrooxidans." Chemosphere **248**: 126080.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