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Exploiting Microalgae for efficient removal of heavy metals: An *in-silico* approach

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ABSTRACT

In recent years, bioremediation has attracted a great deal of attention because of environmental pollutants and their implications for public health and environmental sustainability. In bioremediation, microalgae play a major role in environmental and wastewater treatment techniques. Among environmental contaminants, heavy metals (HMs) are significant pollutants due to their persistence in the environment and their potential to harm ecosystems and human health. Several conventional techniques are available for removing heavy metals, but they are expensive. Microalgae afford an environmentally friendly approach for heavy metal remediation. This review examines the major sources and health effects of heavy metals, including chromium (Cr), arsenic (As), zinc (Zn), cadmium (Cd), Iron (Fe), mercury (Hg), lead (Pb), and Copper (Cu), emphasizing microalgae as a potent tool for heavy metal decontamination. The primary analyses observed microalgal metallothioneins (MTs) and their potential to improve metal sequestration, supported by computational investigations of metal-MT interactions. The study revealed that metal ions with MT proteins binding energies of MT ranged between -16.67 to –3.24 kcal/mol for *P. tenue* and –5.90 to –3.21 kcal/mol for *C. sorokiniana,* –2.86 to –1.41 kcal/mol for *S. platensis,* indicating variable but significant affinity for different metal ions. These results suggest that microalgal MTs play an important role in heavy metal uptake and can be further enhanced using computational and biotechnological techniques. Based on the evidence reviewed, microalgae-based bioremediation systems with MT-enhanced strains are recommended as a potential and long-term solution for heavy-metal removal.

1. Introduction

Growing industrialization and urbanization have exposed ecosystems to a wide range of harmful substances that are hazardous to all living creatures. Significant sources of contamination to aquatic and soil environments include pollutants originating from various industrial processes. Heavy metals are emitted in a variety of forms and concentrations, both during and after industrial production. Heavy metals released from dye companies include mercury and chromium. In agriculture, the use of pesticides, fertilizers, and herbicides releases contaminants like zinc, lead, arsenic, etc. Certain hazardous substances emitted into the nearby environment during mining include lead, arsenic, cadmium, and copper. The cement industry releases cadmium, copper, and zinc and are present in the upper soils. The coal industry also releases pollutants into the environment: copper, arsenic, mercury, chromium,

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lead, nickel, cadmium, and zinc. Water is contaminated by plastics that contain heavy metals. These heavy metals cause liver and cardiovascular diseases, kidney dysfunction, changes in the central nervous system in humans, especially in children, cancers, neurotoxic and nephrotoxic difficulties, reproductive system abnormalities, etc. [1-7]. The overproduction of wastes, along with their discharge into natural water bodies, has a detrimental impact on aquatic ecosystems and the environment. As a result, civilization faces significant challenges regarding the sustainability of our planet [8].

Both conventional and current techniques are employed to eliminate heavy metals from wastewater. Conventional methods, such as electrochemical treatment, osmosis, evaporation, precipitation, and ion exchange, are expensive and require more energy to operate. In order to combat this, current techniques that are more affordable and environmentally friendly, such as biological methods, are employed to remove heavy metals [9]. Phycoremediation is emerging as a crucial platform for cleaning up contaminated systems. Its sustainable features make it far more advantageous than other remediation methods available [10]. Autotrophic microorganisms, known as microalgae, are commonly found in aquatic environments and have shown promise in eliminating heavy metals. Due to their ability to extract nutrients, metals, and organic pollutants, as well as absorb CO2 from the atmosphere, these microbes are also regarded as an efficient bioremediation technique for cleaning industrial wastewater containing heavy metals [11]. Microalgae can grow and develop in the presence of environmental contaminants like heavy metals, phosphate, pesticides, nitrogen, medicines, and dyes. So, they have been proven to be a useful tool for heavy metal treatment in industrial uses [12, 13]. Compared to conventional treatment techniques, microalgae-based bioremediation is the most efficient way to remove harmful substances. This is due to the algal biomass produced by the microalgae, which has a variety of purposes [14]. The unique biological features of microalgae, such as their high photosynthetic efficacy and an easily understood structure, allow them to survive in a variety of challenging conditions, including high salinity, nutritional stress, extreme temperatures, and the presence of heavy metals. Microalgae are especially effective for phycoremediation of harmful heavy metals because of their high binding affinity, huge surface area, and abundance of binding sites. The bioremediation process employing microalgae may safely eliminate toxic metals from the environment. The capacity to generate biofuels and fertilizers from their rapid growth offers microalgae an edge over higher plants. Microalgal biomass can also be exploited to create carbs, proteins, vitamins, and lipids. In addition to protein-rich feed, using microalgae for bioremediation processes may regenerate resources for economic utilization via the biorefinery of microalgal/bacterial biomass, yielding a range of low- and high-value by-products including microalgal plastics, fibers, and fertilizer [15-17]. The objective of the present review is to highlight the phycoremediation of heavy metals and to provide a detailed understanding of the mechanisms by which algae remove heavy metals from wastewater. The efficacy of diverse species via extracellular and intracellular pathways in the biosorption, bioaccumulation, and detoxification of different heavy metals has been thoroughly examined in the context of microalgae. This study will focus on the unique properties of microalgae, such as their ability to absorb and accumulate heavy metals across a range of biological processes and environmental conditions for bioremediation. The in silico technique has the potential to transform bioremediation research through rapid discovery, hypothesis development, and process optimization. The effectiveness of phycoremediation systems in reestablishing environmental health will therefore increase. This review highlights potential obstacles and opportunities in heavy-metal bioremediation techniques utilizing microalgae.

2. Sources of HMs

HMs accumulate in aquatic ecosystems as a result of both natural and human activities. Natural sources include wet and dry atmospheric salt deposition, rock weathering, hydrogeochemical interactions between water and soil, earthquakes, and aquifer leaching into rivers and oceans [18]. The presence of heavy metals in water is affected by hydrogeology, geochemistry, and regional geological factors. In addition to natural contributions, human activities considerably increase heavy metal pollution. Urbanization and industrialization contribute to pollution through household waste discharge, industrial effluents, mining activities, and inappropriate garbage disposal [18]. Agricultural activities, including the excessive use of chemical fertilizers and pesticides, add metals such as cadmium (Cd), arsenic (As), and lead (Pb) to water bodies, increasing pollution levels [19]. Airborne pollutants also contribute to the deposition of heavy metals in water. The combustion of fossil fuels releases carbon dioxide (CO_2), which reacts with water to form acidic compounds that facilitate metal leaching. Sulfur dioxide (SO_2), emitted from coal and petroleum combustion as well as volcanic eruptions, combines with water to produce sulfuric acid, while nitrogen dioxide (NO_2) forms nitric acid, both of which contribute to acid rain and heavy metal mobilization [20]. Mining operations are another significant source, releasing heavy metals into the environment, where they persist due to their non-biodegradable nature and long half-lives. These metals bioaccumulate in aquatic organisms and pose severe health risks to both ecosystems and human populations [21]. The severity of health hazards depends on metal concentration and the duration of exposure, with chronic, low-level exposure leading to long-term toxic effects, including neurotoxicity, carcinogenicity, and organ damage

(Figure 1) [22]. Despite extensive research, gaps remain in understanding the synergistic effects of multiple heavy metals and their long-term ecological impacts, underscoring the need for further studies on mitigation and remediation strategies.

2.1. Toxicity of heavy Metals

2.1.1 Arsenic (As)

Arsenic is a prominent, poisonous heavy metal that contaminates drinking water in many nations, including the USA, Bangladesh, Argentina, China, India, Chile, Southeast Asia, etc. [23, 24].

According to [25], as of 2008, drinking water from hand tube wells with arsenic had been reported in Uttar Pradesh, Jharkhand, Rajnandgaon village in Chhattisgarh, Manipur, Bihar, West Bengal, and Assam states of India. It has been estimated that 50 million people could be at risk of groundwater arsenic pollution. In addition to spreading through mobility and groundwater mining, arsenic pollution of groundwater has far-reaching effects, such as absorption through the food chain, health risks, social disorders, and socioeconomic disintegration [26]. The main industrial operations that lead to anthropogenic arsenic poisoning of water include mining, burning fossil fuels, and smelting non-ferrous metals [27, 28]. Arsenic used for industrial purposes, glass and alloy manufacturing, metal processing, pesticides and sheep dips, fertilizers, leather preservatives, antifouling paints, pharmaceuticals, poison baits, and arsenic-containing pigments are other major sources of contamination. The effect of chronic arsenic exposure from different sources has been investigated in various countries and has been associated with various health effects, such as keratosis, various types of cancer, hyperpigmentation, and vascular diseases [29, 30]. In the environs, arsenic existed in organic and inorganic forms and had various valences [31]. In addition to reducing growth and photosynthesis, arsenic also disrupts thylakoid membranes (Table 1) [32].

2.1.2 Chromium

Chromium is a toxic heavy metal according to the US Environmental Protection Agency. Direct or indirect exposure causes adverse health effects.

It occurs in two valence states, such as trivalent chromium Cr (III), hexavalent chromium Cr (VI), and an uncharged metallic form (Cr) [33].

Chromium is released into the environment through human activities and industrial applications: chrome plating, electroplating, the steel industry, textile manufacturing, alloy making, leather tanning, batteries, dyes, paints, welding, catalysts, and wood preservatives [33-35]. On exposure to a hexavalent form of chromium Cr (VI), diseases such as hemorrhage, epigastric pain, diarrhea, and nausea are observed. Ingestion of Cr (VI) can cause severe health issues like skin, lung, and stomach cancers, kidney problems, tissue neurosis, etc. [35-37]. Of the three existing forms, a hexavalent form of chromium Cr (VI) is a suspected mutagen and extremely carcinogenic. The contrasting trivalent form and metallic form are less harmful [38].

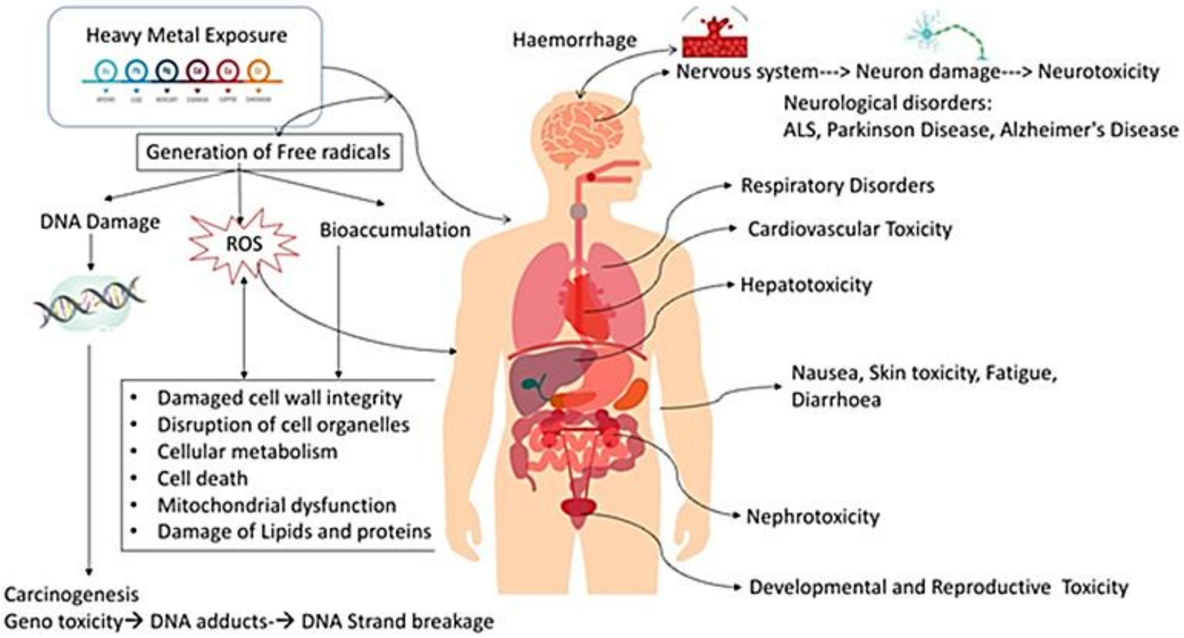


Fig. 1. Chronic toxic effect, poisoning, and health hazards of heavy metal accumulation impact on human health.

Table 1. Heavy Metal Toxicity Mechanisms at the Molecular Level

Heavy Metal	Toxicity Mechanism
Chromium (Cr)	Cr(VI) is a strong oxidizing agent that generates reactive oxygen species (ROS), leading to DNA damage, protein oxidation, and mitochondrial dysfunction.
Arsenic (As)	Mimics phosphate ions, interfering with phosphate metabolism, disrupting ATP production, and inhibiting DNA synthesis.
Cadmium (Cd)	Replaces essential metals like zinc in enzymes, causing protein misfolding and inhibiting antioxidant enzymes such as catalase and superoxide dismutase (SOD).
Mercury (Hg)	Binds to sulfhydryl (-SH) groups in proteins, leading to enzyme inhibition, increased oxidative stress, and neurological damage.
Lead (Pb)	Disrupts calcium homeostasis, impairs synaptic transmission, and interferes with neurotransmitter release in the nervous system.
Zinc (Zn)	While essential, excessive Zn disrupts iron and copper homeostasis and induces oxidative stress at high concentrations.
Iron (Fe)	Catalyzes the Fenton reaction in its free form, producing hydroxyl radicals that cause oxidative DNA and lipid damage.
Copper (Cu)	Undergoes redox cycling, generating oxidative stress and causing lipid peroxidation and cellular damage.

2.1.3 Mercury

Mercury is one of the highly bioaccumulated HMs and can exist in diverse forms. Mercury is ubiquitous and has become inevitable. It is available in three forms (organic, inorganic, and elemental), each with characteristic noxious effects [39, 40]. Mercury can additionally exist as a cation with oxidation states of +1 (mercurous) or +2 (mercuric) [41]. Methylmercury is the most commonly encountered compound in the environment and forms because of the methylation of inorganic (mercuric) forms of mercury through microorganisms found in soil and water [39, 42]. Metallic mercury has been used in instruments such as thermometers, barometers, and blood pressure-measuring equipment[43]. Industrial emissions, ingested through seafood (freshwater and ocean fish), typically contain large quantities of mercury; dental amalgam is another source of inorganic and mercury vapor. Apart from its advantages and usage, it has various adverse effects on human health. Prime routes of exposure to mercury include inhalation and ingestion [41]. Mercury accumulates in the central nervous system (CNS) and is also deposited in body tissues: thyroid, breast, sweat glands, pancreas, prostate, kidneys, muscles, liver, salivary glands, lungs, myocardium, skin, testes, adrenals, and breast milk [44]. Mercury can alter the tertiary and quaternary structures of proteins by binding with sulfhydryl and selenohydral groups, impairing all cellular functions [45]. It also induces lipid peroxidation, oxidative stress, mitochondrial dysfunction, and alterations in heme metabolism. Furthermore, it causes depolarization of the inner mitochondrial membrane, increasing hydrogen peroxide (H_2O_2) formation [44].

2.1.4 Cadmium

Cadmium is a poisonous metal and non-essential element and exposure to high concentrations causes severe damage to human health [46]. Anthropological sources of cadmium vary widely; it is released in industrial refining processes and used in alkaline batteries, the galvanoplasty industry, semiconductor alloys, the textile industry, etc. [47]. Exposure to cadmium at low concentrations damages the liver, kidney, and lungs as a consequence of damaged cell wall integrity due to bioaccumulation. Also, cadmium disrupts the endoplasmic reticulum and mitochondria, which leads to the release of calcium within the cell and apoptosis in a few cells; sometimes autophagy mechanisms are activated and a few elements that are essential nutrients in cell metabolism are replaced [48]. In plants and microbes, it affects growth, morphology, cellular metabolism, and photosynthesis [49]. In addition, cadmium exposure has other injurious effects, including damage to the brain, reproductive failure, tumor formation, and nervous system failure. Meanwhile, exposure to cadmium at high concentrations causes skeletal deformation and spontaneous fractures [50].

2.2. Less toxic Heavy Metals

2.2.1 Iron

Nearly all living things depend on iron for growth and survival [51]. On the surface of the earth, iron is the second abundant metal. It is a crucial component of organisms such as algae, alongside enzymes such as cytochromes (10%), catalase (10%), and oxygen-transporting proteins like hemoglobin (70%) and myoglobin (10%) [52]. The main sources of iron in the surface water are mining activities and anthropogenic activities.

Up to 25% of the body's iron is ferric Fe (III) and is stored in the liver, spleen, and bone marrow as hemosiderin, ferritin, and transferrin. Ferritin can hold up to 4500 atoms of iron per molecule and has a low-capacity protein and high-affinity activity. Transferrin is an iron molecule that contains a high-affinity and low-capacity protein and will transfer two atoms of iron per molecule in plasma, which is another crucial protein in iron homeostasis [53].

Iron can bind to biomolecules as well as molecular oxygen, thereby establishing a link between them. Iron-containing enzymes are also required for other biochemical activities, such as cellular respiration and drug metabolism. Iron deficiency in humans affects nearly half a billion people worldwide and can have a variety of adverse impacts, ranging from anemia to intellectual disability in children. Hemochromatosis is a hereditary illness that requires a high-iron diet and blood transfusions [54]. However, as the Fenton reaction demonstrates, iron is one of the heavy metals that produces hydroxyl radical (OH•) in its free state [55]. A broad range of harmful free radicals are created when ingested iron miscarries attach to protein, and this has a major effect on the amount of iron present in mammalian cells and biological fluids.

The most frequent free radical produced by iron oxidation is the hydroxyl radical (OH•). OH• can damage biological molecules such as DNA, lipids, and proteins [55]. When iron causes oxidative DNA damage, a broad array of molecules is generated from oxidized bases. The oxidized form of guanine (8-Hydroxydeoxyguanosine), which can cause mutations, is the major result of oxidative DNA damage. Bucher and coworkers made the first such observation, demonstrating that iron-generated OH• can damage lipid membranes via a process known as lipid peroxidation [55, 56]. Enzymes like catalase, glutathione peroxidase, and superoxide dismutase essentially neutralize free radicals; at the same time, these superoxide molecules release ferritin from the iron molecule and react with various hydrogen peroxide and superoxide, forming highly harmful free radicals like hydroxyl radical. These hydroxyl radicals are hazardous to health, inactivating certain enzymes and promoting depolymerization of polysaccharides and lipid peroxidation, as well as damaging the DNA strands, which leads to cell death [57, 58].

2.2.2 Copper

Copper is classified as an essential element because it is a requirement for enzymes involved in human biological functions [59]. Mining and milling are the primary contributors to copper contamination. The most common sources of copper exposure in the general population are drinking water and food. Waste dumps, fossil fuels, home wastewater, waste combustion, phosphate fertilizer manufacturing, wood production, and natural sources can all contribute to copper entering the environment. As a result, copper is widely distributed in the environment. Copper intake in the typical population is estimated to be 0.15 mg/day from drinking water and around 2 mg/day from food. On the other hand, minimal evidence suggests that copper biomagnification in the aquatic food chain is moderate. Applications of copper include alloys such as bronze and brass, copper wires, plating, coins, pipes, fertilizer, plates, preservation of wood, maintaining cloth, barrier lotion, and chemical analyses to identify sugar in Fehling's solution. Copper sulfate is used in agriculture to treat mildew and as an algicide in water purification [60, 61]. A multitude of homeostatic mechanisms maintain the body's concentration of copper at a physiologically necessary level. Absorption, cellular efflux, sequestration/storage, intracellular transportation, cellular uptake, and excretion from the body are all part of copper homeostasis [60]. At low levels of consumption, it is a vital component for all known living organisms, including humans and other animals. Toxic effects can occur at much higher concentrations [41]. Copper ions that aid in the formation of reactive oxygen species (ROS) include cupric (Cu^{2+}) and cuprous (Cu^{1+}), which can take part in oxidation and reduction reactions [62]. With the help of biological reductants like glutathione or ascorbic acid, which may catalyze the breakdown of H_2O_2 to yield an OH• radical via the Fenton reaction, Cu^{2+} can be reduced to Cu^{+}.

$$\mathrm{Cu(I)} + \mathrm{H}_2O_2 \leftrightarrow \mathrm{Cu(II)} + \mathrm{OH}\bullet + \mathrm{OH}- \tag{1}$$

The generated OH• radicals can react with a variety of biomolecules. Copper can also cause DNA strand breakage and the oxidation of bases via oxygen-free radicals, according to experiments. Although no in vivo evidence of copper-induced oxidation of low-density lipoprotein (LDL) has been found, in vitro investigations have convincingly established copper-induced LDL oxidation [55]. The role of copper in oxidative-induced stress in neurodegenerative illnesses has been extensively investigated, as copper is an essential component of numerous proteins required for neurological function. Copper has been linked to several neurological diseases, including Alzheimer's, Parkinson's, and amyotrophic lateral sclerosis (ALS) [63]. Alzheimer's disease (AD) is connected with increased oxidative stress, as demonstrated by higher concentrations of Cu, Fe, Al, and Hg in the brain, which can cause the production of free radicals; increased lipid peroxidation and decreased levels of polyunsaturated fatty acids in the AD brain; elevated 4-hydroxynonenal, an aldehyde product of lipid peroxidation in the AD ventricular fluid; and elevated levels of protein and DNA oxidase in the AD ventricular fluid [64].

2.2.3 Zinc

According to the International Agency for Research on Cancer (IARC), zinc is classified as a Group-IV carcinogenic element, which is probably not carcinogenic [44]. Zinc is a bluish-white, shiny metal belonging to the transition elements, existing in the oxidation state of +2 with the atomic number (Z) 30 [21]. Zinc can enter the air, soil, and water through several anthropogenic activities, including the purification of zinc-lead, mining, steel production, burning coal, cadmium ores, and burning wastes. Sludge and fertilizers used in agriculture can increase soil levels. This trace mineral acts as a helper molecule that aids almost 300 enzymes in our body, such as DNA polymerases, alkaline phosphatase, Zn-superoxide dismutase, RNA transcriptase, and also in nucleic acid synthesis and cell proliferation [65, 66]. The brain, bones, muscles, prostate, liver, kidneys, and eyes all contain high concentrations of zinc. According to in vitro tests, ZnO NPs selectively kill cancerous cells. Because ZnO NPs selectively target cancer cells, they have 28–35 times the selective toxicity of normal cells against malignant cells. This makes them useful in an in vivo system [67]. The zinc binds to cysteine and histidine residues in particular peptides, forming a tertiary structure that binds to specific DNA sequences in promoter gene areas [68]. The zinc finger, the most common zinc motif, and the zinc thiolate cluster are among the configurations. Other biological functions include increasing the affinity of growth hormone for its binding receptors, modulating synaptic transmissions [69]. Zinc also interacts with specific locations on ionotropic neurotransmitter receptor proteins and induces the release of neurotransmitters. Zinc is also required for immune system function as well as for the maintenance and integrity of cellular components (molecules and or membrane stability) [21]. Zinc deficiency in humans has been implicated in several acute symptoms. In humans, it has been linked to male hypogonadism in adolescents, poor appetite, cell-mediated immune dysfunction, oligospermia, hyperammonemia, and mental lethargy, as well as various growth-related effects [70]. When zinc is consumed in zinc-fortified foods or water, it enters the body through the digestive tract. Zinc can persist in the bones for days after exposure and is eliminated via urine and feces [71]. Zinc in the lumen can harm the brush border membrane, increasing zinc absorption, as it binds to cell protein and other ligands non-specifically [61]. Zinc cytotoxicity is triggered by the intracellular release of zinc ions and the subsequent formation of reactive oxygen species (ROS) [21, 66]. In sum, both environmental and human-made activities lead to the presence of heavy metals in water bodies, yet anthropogenic factors remain the main source of contamination. Identifying those reasons is important for maintaining targeted assessment, maintaining regulation, and establishing mitigation and remediation techniques that effectively minimize HM contamination.

3. Role of Microalgae for heavy metal removal

Microalgae require trace amounts of heavy metals (HMs) such as Cu, Co, Mo, Zn, Fe, and Mn for enzymatic and cellular metabolism, but they are poisoned by As, Cr, Cd, Pb, and Hg [72, 73]. Based on hormesis phenomena, mild toxic heavy metals are promoting microalgae growth and metabolism [74].

Because of their reactive groups and active binding sites, microalgae can combine with wastewater contaminants to produce complexes (Figure 2, Table 2). It affects flocculation and eventually diminishes the contents of both the total dissolved solids (TDS) and total suspended solids (TSS) [24, 75].

Microalgae produce antioxidant enzymes like glutathione reductase, superoxide dismutase (SOD), catalase, peroxidase, and ascorbate peroxidase, as well as non-enzymatic antioxidants like ascorbic acid (ASC), glutathione (GSH), cysteine, carotenoids, and proline to combat free radicals released by heavy metals during adsorption [76]. By breaking down the superoxide anion into oxygen molecules and hydrogen peroxide, SOD serves as the main line of defense against it [77]. Catalase reduces hydrogen peroxide to water and oxygen molecules. Glutathione and ascorbic acid are significant antioxidants produced by microalgae that help to reduce free radicals and reactive oxygen species (ROS) [24, 78]. Based on the degree of metabolic dependency, metal-aggregated bioprocesses commonly fall into two types. It depends on the microorganisms that eliminate metals from solutions which comprise (i) extracellular accumulation, based on usage of viable microorganisms; (ii) cell-surface sorption, which depends on both living and dead microorganisms; and (iii) intracellular accumulation, influenced by microbial activity [79]. The ions of heavy metals are generally captured in the cellular structure and then biosorbed to binding sites existing in the cell structure. This kind of absorption is termed “biosorption” or “passive uptake” and is based on a self-regulating biological metabolic cycle [90].

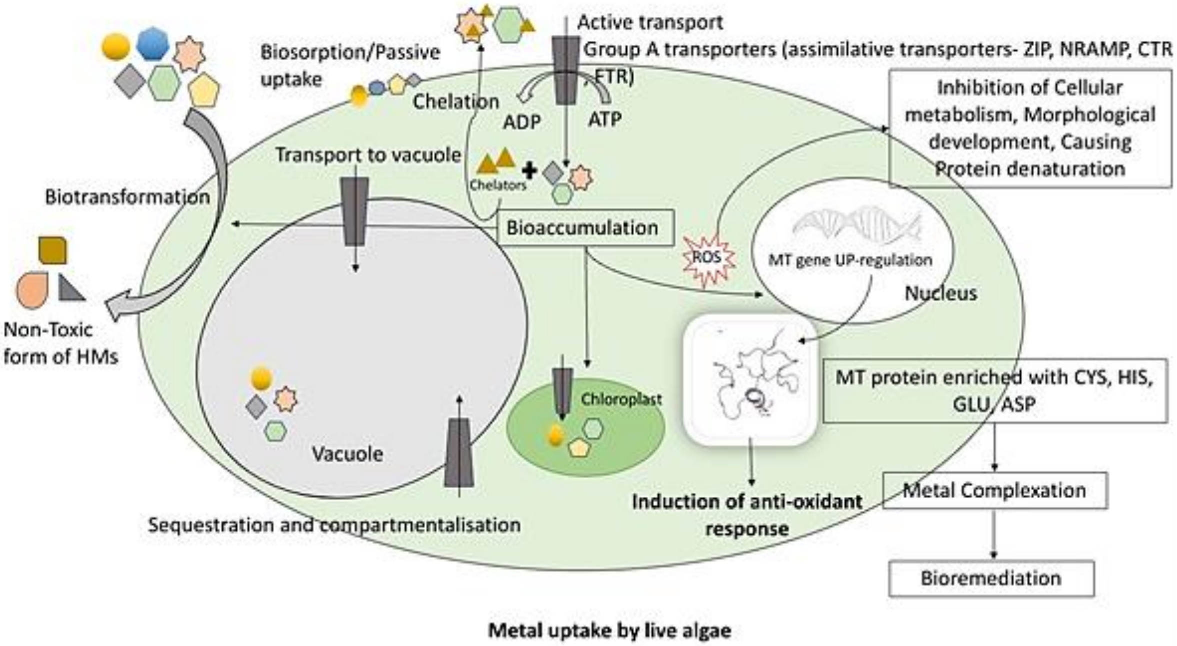


Fig. 2. Mechanism of heavy metals uptake by live microalgae.

Table 2. Various Microalgal Species and their Bioremediation Capabilitis for Heavy Metal Removal

Microalgal Species	Heavy Metals Removed	Bioremediation Mechanism	Refs.
Chlorella vulgaris	Cd, Pb, Cr, Zn, Cu	Biosorption, bioaccumulation, enzymatic detoxification	[9,80]
Scenedesmus obliquus	Cr, Ni, Cu, Zn	Surface adsorption, intracellular accumulation	[80]
Spirulina platensis	As, Pb, Hg	Chelation with proteins, metal sequestration in vacuoles	[81]
Pseudokirchneriella subcapitata	Pb, Cd, Cu	Active uptake, intracellular complexation	[82]
Dunaliella salina	Cu, Zn, Hg	Antioxidant response, enzymatic detoxification	[39, 83]
Chlamydomonas reinhardtii	Cr, Ni, Pb, Cu	Metal-binding proteins, oxidative stress response	[84]
Porphyridium sp.	Hg, Pb, As	Extracellular polymeric substance (EPS) production	[85]
Anabaena flos-aquae	Cr, Pb, Cd, As	Bioaccumulation, extracellular binding	[80]
Synechococcus elongatus	Ni, Cu, Zn	Active transport, metallothionein expression	[85,86]
Oscillatoria limnetica	Cd, Pb, Cr	Metal sequestration via protein binding	[87]
Phormidium sp.	Zn, Cu, Pb	Biosorption through exopolysaccharide production	[88]
Botryococcus braunii	Cr, Ni, Pb, Cu	Cell wall adsorption, lipid-bound metal detoxification	[89]
Navicula sp.	As, Pb, Cd	Biosorption through silica-based cell walls	[54]

HMs can enter the cell during the cell metabolic cycle, crossing the cell membrane; this method of metal uptake is defined as "active uptake." The term "bioaccumulation" refers to the metal uptake that occurs in both active and passive mechanisms. On the other hand, a few observations indicated that dead (otherwise chemically treated) cells can accumulate HM ions to the same proportion as developing or resting cells. The adsorption that results is quick, widespread, and frequency selective [85]. The metal uptake by the live microalgae has several strategies. These mechanisms include (i) the character of the algal cell wall in binding heavy metals due to its intrinsic composition. Proteins, polysaccharides, and lipids make up the majority of the microalgal cell wall, and each of these functional groups (e.g., hydroxyl, phosphate, carboxyl, sulfhydryl, and amino) has a strong affinity for binding metal cations through counter-ion interactions and transmits a net overall negative charge to the cell surface [47]. Chojnacka et al., 2005 observed that cell wall components of algae—including polysaccharides, proteins, teichoic acid, teichuronic acid, and peptidoglycan—carry charged groups such as phosphate, hydroxyl, amine, and carboxyl groups. The algal cell wall's amphoteric characteristics are due to the presence of anionic and cationic sites. Functional groups are vital for HM uptake by both living and non-living microalgae. Additionally, these groups are either protonated or deprotonated based on pH [85]. (ii) The plasma membrane accommodates membrane metal transporters, which play the foremost role as a defense in the alteration of the functioning of a biological system by environmental factors (cellular perturbations). Metal ions are transported into the cytoplasm via Group A transporters like ZIP (Zrt-, Irt-like Proteins), NRAMP (Natural Resistance Associated Macrophage Proteins), CTR (Cu transporter), and FTR (Fe transporter) [91]. These Group A transporters originate in the vacuole membrane and play a similar role as assimilative transporters, where the metal source is an intracellular storage compartment rather than the external environment. Heavy metal concentrations in the cytoplasm are reduced by FPN (FerroPortiN), P1B-type ATPases, and Ca (II)-sensitive Cross-Complementer 1/ Vacuolar Iron Transporter 1 families and Group B transporters (Cation Diffusion Facilitator) [92].

There are several other methods that microalgae have adopted, including ion exchange, adsorption, metallothioneins, sequestration, polyphosphate bodies, compartmentalization in the vacuole, and sequestration in the mitochondria and chloroplasts. However, non-viable microalgae have been recognized for their potential to remove HM ions promptly through simple biosorption, with minimal toxicity issues. Their sorption capability is just moderately lower than that of their living [85]. However, non-viable microalgae have long been known for their potential to remove HM ions promptly through simple biosorption, with minimal toxicity issues. Their sorption capability is just slightly lower than that of their living counterparts.

It has many advantages: higher efficiency and affinity, eco-friendliness, no nutrient and growth media requirements, being dynamic over a wide range of physiological conditions, ease of investment and low operational costs, and feasible recovery [85]. During laboratory-scale experiments, the effectiveness with which microalgae remove HM is determined by the microalgal species, the characteristics and concentration of the metal ion, and the culture period [93]. For instance, wheat bran, rice husk, sugarcane bagasse, fruit/vegetable waste, soybean hulls, corncobs, almond shells, eggshells, neem barks, black tea waste, papaya seeds, sawdust, and many more have been included in HM adsorption. All these agricultural wastes have a significant percentage of heavy metal adsorption [94].

4. Mechanism of toxicity

Heavy metals such as mercury, arsenic, copper, chromium, zinc, cadmium, and lead are among the most toxic and essential components of the environment in which we live. Heavy metals are persistent environmental contaminants that occur naturally in the Earth's crust and have numerous detrimental effects on ecosystems. A variety of biological processes may be adversely affected by heavy metals, which can enter the body through several routes, including ingestion of contaminated food or water, inhalation of contaminated air, and cutaneous absorption.

4.1. Mercury

Microalgae provide an economical way to separate mercury ions from diluted solutions by using biosorption processes to effectively remediate mercury and other heavy metals. The advantages of microalgae-based treatment over traditional techniques include their rapid growth, remarkable specialization, environmental friendliness, cost-effectiveness, and high efficiency in removing heavy metals without releasing any secondary pollutants [86, 95]. The process of biosorption is considered passive. One specific application of the sorption process is heavy metal biosorption. Biosorption is a feature of biomass that involves special biomolecules that may bind and concentrate particular ions in aqueous solutions. The primary reason for the biosorption process is the affinity between the biosorbent and the adsorbate. Heavy metals adsorb onto the surface of microalgae via a variety of processes, such as ion exchange with the microalgae's cell wall ions, covalent bond formation with the ionic cell wall, and interaction with positively charged cations found in the exopolysaccharides of the organism. Furthermore, the biosorption process depends on operating parameters like temperature, pH, metal type, and concentration. The presence of various binding groups, including sulphuryl, hydroxyl,amine, carboxyl, carbohydrate, phosphoryl, sulfate, imidazole, phosphate, and other functional groups, on the surface of algal cells is thought to be responsible for the ability to biosorb heavy metal ions. Bioaccumulation is thought to be an active process. On the other hand, the accumulation of heavy metals inside the cell usually happens significantly more slowly. It comprises diffusing and attaching to internal binding sites of proteins and peptides, such as glutathione (GSH), metal transporters, oxidative stress-reducing compounds, and phytochelatins, after active metal transport across the cell wall into the cytoplasm [96,97]. Before harming the environment, the toxin must be detoxified within the permitted limits. Algal cell walls contain a variety of biomolecules, including proteins, lipids, and carbohydrates, that aid in the interaction with heavy metals. The functional groups that make up these biomolecules include oxygen, nitrogen of the peptide bond, histidine group, phenolic, amino, thiol, sulfhydryl, imidazole, ether, phosphate, carboxyl, phosphate, hydroxyl, sulfate, phosphoryl, and amide moieties. These groups act as catalysts for directing the formation of bonds with metallic ions and also facilitate the adsorption of metal ions in an algal cell.

The varying adsorption capacities of different algal strains may result from variations in the distribution and amount of the polysaccharides and proteins that make up their cell walls [87]. Two cyanobacterial strains, *Spirulina platensis* and *Aphanothece flocculosa*, have been examined by [98] for their ability to absorb mercury. The strains removed 98% of the mercury with a primary concentration of 10 ppm at pH 6 [91]. The absorption capacity of two brown algae, *Macrocystis pyrifera* and *Undaria pinnatifida*, which that are members of the Phaeophyta class and Laminariales order, was investigated by [99]. *U. pinnatifida* (4.4 L/mmol) has shown a higher absorption affinity for mercury uptake than *M. pyrifera* (2.7 L/mmol); however, the uptake of mercury was significantly reduced when other opponent heavy metals, such as Cd (II), Ni (II), and Zn (II), were present. Using Fourier transform infrared spectrometry analysis, this study found that Hg (II) can bond with S=O (sulfonate) and N–H (amine) functional groups. Additionally, *M. pyrifera* and *U. pinnatifida* were found to be the most effective algae for mercury remediation [99].

4.2. Arsenic

The International Agency for Research on Cancer (IARC) has identified arsenic (As) as one of the world's leading carcinogens. Prolonged exposure to arsenic can result in arsenic poisoning.

Algal sorption of arsenic holds great promise for bioremediation due to its high removal efficiency and sustainability. Numerous phytoplankton and cyanobacteria species can withstand high concentrations of aqueous As species, making them potential candidates for As environmental cleanup programs. In order to detoxify, algae have developed a number of metabolic processes [100, 101]. Arsenate (As^{V}) enters cells by phosphate transporters in prokaryotic (like cyanobacteria) and eukaryotic (like Chlorella sp.) algae, whereas arsenite (As^{III}) is absorbed across the plasma membrane by aquaglyceroporins and hexose permeases. Because PO4 $^{3-}$ and AsO4 ^{3} share molecular similarities, arsenate functions as a competitive inhibitor of phosphate. According to numerous research studies, the microalgae *Chlorella salina, Chlamydomonas reinhardtii*, and *Skeletonema costatum* dramatically reduced their As^{V} uptake when phosphate was added. Thus, raising the quantity of PO4 $^{3-}$ in the environment could decrease the uptake of arsenate and the toxicity that results in algae [102]. In the accumulation and sequestration of algae in a more efficient manner, it can change the As(V) form into As(III) inside the cell; however, As(III) conversion to organic As form is extremely slow, probably because As(III) exudes to the external culture medium so quickly [103].

Algae have developed a variety of metabolic pathways to detoxify As. There may be additional As(V) transport mechanisms in algae than As absorption via phosphate channels, as previous research (though partial) suggested phosphate independent uptake of As(V) by algae [100]. Cell surface adsorption, intracellular As(III) oxidation, As(V) reduction, thiol (-SH) complexation, and sequestration within vacuoles are some of the processes of As detoxification by microalgae that have been shown in several studies [100]. Huang et al. reported that after exposure to the three As(V) concentration gradients of 100 µg/L, 500 µg/L, and 1000 µg/L, the algae's As contents on the tenth day were 19.3 mg/kg, 49.5 mg/kg, and 145.17 mg/kg, respectively. The As contents were subsequently reduced. Therefore, it was possible to promote the absorption of As(V) during the first 10 days of exposure, regardless of how much As was absorbed in the algae, when the arsenic concentration in the exposed medium was below 1000 µg/L. However, the algal cells eventually died 10 days later due to arsenic toxicity. The findings showed that exposure duration, rather than the concentration of As in the solutions, was the primary determinant of As toxicity [101].

According to Wang et al. (2015), As biotransformation in algae can take place by As(V) uptake, which then results in As(V) transformation to As(III) through reduction and ensuing formation of methylated species [89]. By reducing arsenate and complexing with thiol peptides in cells, it is commonly believed that many algae may detoxify As(V) to As(III) and methylarsenic [104]. However, because minimal As(V) was digested by algal cells, As detoxification was less likely to occur due to As bioaccumulation and subsequent methylation.

4.3. Zinc

Zinc is the fourth most common ecotoxic metal that pollutes both aquatic and terrestrial environments. The use of microalgae to remediate wastewater is increasing worldwide. The polysaccharidic matrix in which most microalgae species are contained allows metal ions like zinc to adhere to them. Ibout et al. reported the entering of zinc into microalgae cells and internalization into the cytoplasm and cytoplasmic organelles in addition to the extracellular uptake mechanism. In actuality, zinc ions may be bound to particular intracellular substances, like phytochelatins or metallothioneins III, or they may be carried into cellular compartments, primarily vacuoles [84, [105]. Certain species of microalgae produce metal-binding proteins, such as metallothioneins, which facilitate the movement of metals across the cell membrane, allowing them to collect heavy metal ions. These proteins, commonly referred to as phytochelatins, are also essential for metal detoxification and metal ion homeostasis.

In response to the harmful effects of heavy metals, several algae species produce phytochelatins, internal metal chelators that detoxify metals by chelating excess elements into innocuous molecules. Furthermore, in certain algae species, such as the blue-green algae *Anabaena flos-aquae* and the green algae *Scenedesmus acutus*, cellular polyphosphate granules function as metal detoxifiers [80]. Zhou et al. reported that *S. obliquus* and *C. pyrenoidosa* demonstrated strong zinc accumulation characteristics, with growth being inhibited only at comparatively high concentrations. The high level of resistance to these metals raises the prospect of employing these two microalgae in freshwater bioremediation procedures for zinc pollution [80]. The findings demonstrated that the metals adsorbed at the surface of the algae cells were significantly more than those accumulated intracellularly after eight days of exposure to zinc. This is in accordance with the findings of earlier research on the marine alga *Dunaliella salina* and the freshwater alga *S. subspicatus* [106, 83]. These findings show that non-metabolic absorption is crucial to algae's ability to remove metals.

4.4. Copper

Developing a biotic ligand model for accurately predicting Cu toxicity to microalgae requires an understanding of the mechanism of action of Cu and how it causes toxicity in algal cells. Determining the intracellular key biotic ligand and incorporating mechanistic processes, such as toxicokinetic and toxicodynamic processes, might enhance the prediction of metal toxicity for microalgae [107]. The species-specific character of copper internalization and detoxification following Cu binding to thiols and phytochelatins has been brought to light by mechanistic investigations using marine microalgae. The marine diatom *Ceratoneis closterium* (previously *Nitzschia closterium*) cells exposed to Cu showed reduced levels of thiols, and Stauber and Florence observed that cell growth was restored after adding sulfhydryl compounds to the bioassay test media. In the algal cytoplasm, they suggested that Cu attaches to reduced glutathione (GSH) and that GSH may reduce Cu(II) to Cu(I) and bind as a Cu(I)-sulfur-complex, oxidizing the reduced glutathione to oxidized glutathione (GSSG) [108]. When Cu(II) exposure caused significant inhibition of cell division and an increase in the oxidized pool of glutathione in *C. closterium*, Smith et al. provided evidence in favor of this theory. A decrease in the rate of growth of an algal population is a common indicator of inhibition of algal cell division, which can result from any disturbance of the GSH:GSSG ratio that affects mitotic spindle formation [109, 110]. These mechanisms could be extracellular activities, such as metal ion binding to cell exudates or ligands or physical exclusion, brought on by decreased membrane permeability [106]. Metal-binding proteins, such as phytochelatins (which contain many sulfur-rich cysteine residues), are also synthesized inside cells. Cell exposure to Cu can also change enzymes that can scavenge harmful reactive oxygen species, such as catalase and superoxide dismutase. Due to their high rate of cell division, cells may also efficiently dilute intracellular Cu concentrations or remove Cu through efflux processes [111]. Copper detoxification mechanisms can include transport into the cell and sequestration/storage by proteins and other molecules like metallothioneins and phytochelatins, either within the cytosol or internal compartments, as well as binding to the cell wall and excreted polymers (mucilage). There are two types of the redox-active metal copper: Cu^{2+} and Cu1+. Copper can create coordination bonds with phosphates and/or various N and S ligands in microalgae (such as glutathione, phytochelatins, and plastocyanin). Furthermore, too much copper can cause cellular damage and functional loss by substituting other necessary metals with structural or enzyme cofactor roles [112, 113].

Compared to green algae *Tetraselmis sp.* (72-h IC50 47 g Cu L−1) and *Dunaliella tertiolecta* (72-h IC50 530 g Cu L−1), the diatom *Phaeodactylum tricornutum* exhibited greater reactivity to copper, with an IC50 of 8.0 g Cu L−1. Compared to *P. tricornutum* (0.23 ± 0.19 × 10−13 g Cu cell−1) and *D. tertiolecta* (0.59 ± 0.05 × 10−13 g Cu cell−1), *Tetraselmis sp*. had significantly higher intracellular copper (1.97 ± 0.01 × 10−13 g Cu cell−1) at these IC50 concentrations, indicating that *Tetraselmis sp*. efficiently detoxifies copper within the cell [114]. When exposed to copper, *C. sorokiniana* "undertakes" a quick external metamorphosis by releasing mucilage that contains polymeric phosphate accumulations, altering the outer layer of the cell wall's mechanical and metal-binding characteristics. Cu significantly altered the mechanical characteristics of supramolecular lipid structures as well as the general lipid profile in *C. sorokiniana*. Cu^{2+} coordination to polyphosphates in pentacoordinated square-pyramid geometry is the primary pathway for copper detoxification and buildup; however, reduction to Cu1+ also appears to occur. It's possible that the mucilage and cell wall may play a significant part in buffering large levels of external copper [115].

4.5. Cadmium

Cadmium contamination in aquatic environments is a major environmental issue that leads to the deterioration of both food and water quality. In response to harmful metals such as cadmium, microalgae alter their metabolic composition and produce stress proteins, namely metallothioneins and phytochelatins [115]. The biosorption process is rapid and nonmetabolic. It is primarily controlled by the negative charge of the functional groups on the cell surface because of the presence of proteins, polysaccharides, and phenolic acids that are rich in hydroxyl (−OH), amino (NH2), phosphate (PO4), sulfhydryl (−SH), and carboxyl (−COOH). The metabolism-dependent process of bioaccumulation is only possible in living cells. Transcriptomic analysis can identify the genes involved in the bioaccumulation process. Group A transporters enable the movement of heavy metals from the membrane into the cytosol, while Group B transporters help the metal localize [116]. There have been reports of several Group A transporters being elevated under Cd, including the Cu TRansporter (CTR), Zrt-, Irt-like proteins (ZIPs), Fe TRansporter (FTR), and natural resistance-associated macrophage proteins (NRAMPs). Group B transporters, including the cation diffusion facilitator (CDF), P1B-type ATPases, ferroportinN (FPN), and

Ca (II)-sensitive cross complementary 1 (ccc1) or vacuolar iron transporter 1 (VIT1) families, then aid in the localization of the Cd into internal organelles. Glutathione, phytochelatins, and metallothioneins have also been shown to be increased in microalgae exposed to metals [117].

Cadmium (Cd) in their surroundings is tolerated and detoxified by microalgae through a variety of processes. These strategies include the efflux of excess transition metal ions from the cytoplasm and the movement of cadmium to vacuoles, which is made possible by phytochelatins. Furthermore, microalgae can isolate heavy metals from the cytoplasm by sequestering them into vacuoles or other organelles [118]. Biosorption and bioaccumulation are important detoxifying processes used by microalgae. Additionally, they have the ability to actively detoxify metal ions, sequester them intracellularly, and immobilize them extracellularly. Through complexation, reduction, ion exchange, surface precipitation, and electrostatic interactions, the cell wall and extracellular polymeric substances (EPS) contribute to the adsorption of harmful metals.

Amino acids, chelating peptides, and organic acids can chelate heavy metals in the cytoplasm, and the cells' antioxidant systems can control oxidative damage brought on by reactive oxygen species (ROS) [119]. When exposed to 15 ppm Cd, the microalga eliminated almost 88% of the Cd and managed the Cd stress with an IC50 value of 18.5 ppm. In addition to chelation by EPS and adsorption on the cell wall, Cd accumulation within cells was facilitated by absorption via Ca channels. *Coccomyxa sp.* rewired its redox system to withstand the Cd stress by causing the accumulation of lipids, carbohydrates, and a swarm of enzymatic and non-enzymatic molecules [120]. It was demonstrated that *Tetratostichococcus sp.* P1 could eliminate Cd from the medium. Compared to previous studies employing living cells under acidic conditions, Tetratostichococcus sp. P1 demonstrated a superior Cd^{2+} removal yield, indicating its strong potential as an efficient cadmium biosorbent in such environments. Microalgal strains cultivated at neutral pH or acidophilic microalgae at acidic pH have been shown in the majority of studies to collect or remove Cd [121].

4.6. Chromium

The primary applications for chromium compounds were offset printing and electroplating; these processes are not biodegradable and can cause cancer if they are allowed to accumulate over time. The issue of chromium contamination must be resolved immediately as Cr(VI) poses a greater threat to human health and the environment [122]. Microalgae, which use processes such as adsorption and absorption, have shown promise as agents for reducing Cr(VI) infection. Adsorption occurs when Cr(VI) ions remain on the surface of microalgal cells, whereas absorption occurs when the same ions are taken up by the cellular structure. Developing successful strategies to utilize the unique capacities of microalgae in remediation efforts requires an understanding of the importance of these processes. Through biosorption and bioaccumulation, microalgal biomass can be employed as a bioadsorbent to remove Cr. Both Cr6+ and Cr3+ can be eliminated from polluted aquatic environments by algae. Consequently, there has been a growing interest in removing chromium from algae. Both living (active biomass) and dead (inactive biomass) algal cells can undergo metal sorption, although the processes are distinct [123, 124].

Both active biosorption (bioaccumulation) and passive biosorption (biosorption) are types of biosorption. Active biosorption uses live microalgae, while passive biosorption uses dead cells or materials. The cell wall of microalgae is mostly composed of polysaccharides, lipids, and proteins. These components include a variety of functional groups, including amino (-NH2), carboxyl (-COOH), hydroxyl (-OH), sulfate ($-SO_42-$), sulfhydryl (-SH), phosphate (-PO3), and sulfonate (-SO3−), which contribute to the total negative charge to the cell surface [125]. Microalgae absorb Cr (VI) and convert it to Cr (III) with the help of chromate reductases. Through complexation with metallothioneins, enzymatic reduction to Cr (III), adsorption on cell walls, and ROS detoxification, microalgae can remove Cr (VI). The elimination of Cr(VI) utilizing both living and dead microalgal cells was studied by Yen et al. (2017). In addition to adsorption, they discovered that living microalgae cells also removed Cr through biological processes such as the enzymatic conversion of Cr(VI) to Cr(III) [126].

The greatest removal efficacy (100%) of Cr(VI) was demonstrated using microalgal biochar [127]. According to Pagnanelli et al. (2013), *Chlorella vulgaris* removed Cr(VI) through a biosorption–bioreduction process, in which the bulk of the biosorbed Cr(VI) was converted to Cr(III) on the microalgae's cell wall [128]. Therefore, by converting Cr(VI) into non-toxic Cr, microalgae can lessen its harmful effects.

In sum, HMs have long-term, cumulative, and multisystemic effects on health. These risks can impact several physiological systems through complex biological mechanisms, rather than being restricted to a single organ. Effective solutions to address HMs must be implemented collectively in order to maintain the environment and public health.

Environmental regulation must be strengthened, industrial emissions of heavy metals (HMs) must be carefully monitored, cleaner production technologies must be promoted, and the production and release of HMs in manufacturing processes must be reduced. However, it is crucial to raise public awareness of the risks associated with heavy metals (HMs), promote eco-friendly lifestyles, minimize the use and disposal of products containing HMs, and ensure that waste is properly classified and recycled.

5. Removal of heavy metals by microalgae

Contamination of water bodies by HMs is a major environmental issue worldwide. Since HMs are not biodegradable, they can accumulate in food chains and produce a variety of harmful symptoms in exposed living things, including humans. Phycoremediation, or the use of microalgae's ecological activities in the treatment of wastewater contaminated with heavy metals, is one of the most advised methods because of its efficacy, affordability, and ecological aspect.

5.1. Removal of Mercury

Algae found in contaminated areas are classified as either metal-tolerant or metal-resistant, according to Stokes PM (1983). Cu^{2+}, Cd^{2+}, Pb^{2+}, and Zn^{2+} tolerance and resistance are found in some green algae species. Even at the minor concentrations (5 mg/ml), Hg^{2+} appeared to be very harmful to the three algae species that include *Phormidium ambiguum* (Cyanobacteria), the most sensitive, followed by *Scenedesmus quadricauda* (Chlorophyta) and *Pseudochlorococcum typicum* (Chlorophyta). They achieved a removal rate of 70% of the initial metal content in the first 0.5 hours of contact time. Hg^{2+} inhibited chlorophyll a biosynthesis at moderate concentrations (5-10 mg/ml); the destruction of thorough algal cells occurred at values above 20 mg/ml. When the Hg content in the solution was 40 mg/l, *Spirogyra hyaline (*Microalga) achieved the maximum biosorption in 120 minutes. *S. hyalina* was capable of eliminating 39.212 mg/g Hg from the environment. *Spirulina subspicatus* was also evaluated but found to be ineffective, removing only 1.4 mg/g, representing 60% of the primary metal ion (Table 3)[129, 130]. The algal cell contents of phycocolloids, sulphate, phosphorus, and nitrogen may be responsible for these biosorption activities. However, the acquired outcomes suggested that the highest bio-removal capacity (Cd, Pb, and Hg) occurred after 30 minutes of the trial, and this capacity dropped gradually over the next 24 hours of HM interaction. These could be attributed to the algal cell's internal and external equilibrium. Secondly, it could be due to the breakdown of interactions between the algal cells and metals by microorganisms, such as fungi and bacteria, that grew during the experiment.

When *P. typicum* was treated with Hg+, definite observable changes occurred. All treated cells had an electron-dense layer on their cell surfaces as well as a buildup of starch surrounding the pyrenoids.

Table 3. Role of Microalgae for Bioremediation of Heavy Metals

Heavy Metal	Properties of heavy metal	Sources of heavy metal	Health Hazards	Bioremediation through Algal species		Refs.
				Organism	**Metal uptake (mg/g)**	
Chromium (Cr)	Density:7.15gm/cm^3 Hard, Shiny, Steel grey, Fairly Active metal, Reacts with most Acids.	Chrome plating, electroplating, steel industry, textile manufacturing, alloy making, leather tanning, batteries, dyes, paints, welding, catalysts, wood preservative.	Cancers of skin, Lung and stomach, kidney problems, Tissue neurosis.	*Chlorella spp* *Chlamydomonas reinhardtii* *Spirulina spp*	9.62 25.6 143	[131] [132] [133] [134]
Cadmium (Cd)	Density: 8.69g/cm^3,Found frequently in combination with zinc, Silvery bluish tint metal.	Industrial process, alkaline batteries, galvanoplasty industry, semiconductor alloys, textile industry, etc	Damage to brain,Reproductive failure, Tumor formation,Nervous system failure.	*Chlamydomonas reinhardtii* *Chlorella sp. HA-1* *Desmodesmuspleiomorphus (L)* *Scenedesmus obliquus* *Planothidiumlanceolatum*	5.75 21.6 61.2 11.4 275.51	[135] [136] [137] [138]
Arsenic (As)	Density:5.75g/cm^3,Found as 3 allotropic forms, bright silvery-grey in color, Brittle.	Mining, smelting of non-ferrous metals, burning of fossil fuels, Glass and alloy manufacturing, metal processing, pesticides and fertilizers, pharmaceuticals, sheep dips, leather preservatives, arsenic-containing pigments, antifouling paints, and poison bait	Hyperpigmentation, Keratosis, Cancer,Vascular diseases, Brain damage.	*Hydrodictiyon reticulum* *Diatoms* *Pithophora sp.* *Phormidium sp.* *Oscillatoria sp.*	0.76	[139] [140] [26]
Mercury (Hg)	Density: 13.5336g/cm^3, rare to find in a natural	Industrial emission, seafood(freshwater,	Damage to nervous, digestive and immune system.	*Pseudochlorococcumtypicum* *Scenedesmus acutus IFRPD 1020*	15.13 20 35.71	[141] [52]

	state, at room temperature it's a silvery liquid metal.	ocean fish) plates and dental amalgam.	antibiotic resistance, mental retardation, reproductive disturbance.	*Spirogyra hyaline* *Spirulina spp.* *Chlorella vulgaris BCC 15*	1.34 18	[89] [142] [143] [142]
Zinc(Zn)	Density: 7.34g/cm^3, Silvery-White metal with a blue tinge, Tarnishes in air, Essential element.	Mining, purifying of zinc-lead, and cadmium ores, steel production, coal burning, and burning of wastes. Sludge and fertilizers	Nausea and vomiting, stomach pain, Diarrohea.	*Euglena gracilis* *Pithophoraodeogonia* *Stigeoclonium tenue* *Spirogyra insignis* *Hydrodictyonreticulatum*	7.5 8.98 0.77 21.1 3.7	[144] [145] [146] [147] [148] [146]
Copper (Cu)	Density: 8.96 g/cm^3 Reddish gold color, Essential element, Good conductor of heat and electricity.	Drinking water and food. Waste dumps, home wastewater, fossil fuel and waste combustion, wood production, phosphate fertilizer manufacturing, and windblown dust, native soils, volcanoes, decaying flora, forest fires.	GI mucosal ulcerations and bleeding, central-nervous-system (CNS) manifestations, including dizziness, headache, convulsions, lethargy and coma.	*Chlorella vulgaris* *Spirulina spp.* *Porphyridium purpureum* *Synechocystis spp.* *Hydrodictyonreticulatum*	34.89 100 0.27 23.4 8.72	[144] [133] [149] [150] [146] [151]
Iron(Fe)	Density:7.8g/cm^3, it is known to exist in four distinct crystalline forms, iron rusts in damp air, it dissolves	Fossil fuel combustion, dry coke cooling	Nausea, vomiting, abdominal pain, and diarrhea, multi-organ failure.	*Chlorella vulgaris* *Microcystis sp.*	24.52 0.03	[144] [24]
Manganese(Mn)	Density: 7.3g/cm^3 5th most abundant metal, Essential trace element, Versatile, Exists in 3 oxidation states.	Alloys, Fertilisers, Fireworks, Pesticides, Cosmetics.	Hypotention, Dullness, Weakness, Anxiety.	*Chlorella vulgaris* *Spirogyra spp.*		[54] [152] [61]
Nickel(Ni)	Density:8.9g/cm^3 Sillvery metal, resists corrosion at high temperature, High amount of Ni come from meteorites.	Plating, Jewellery, Coins, Welding, Rocket engines.	Lung embolisms, Asthma, Allergic Reactions, Respiratory failure, Heart disorders.	*Asparagopsis sps* *Chlorella vulgaris* *Spirogyra hyaline* *Sargassum sps* *Scenedesmus*		[148] [153] [154] [155] [156] [157]
Lead	Density: 11.3g/cm^3 Dull silver grey metal, Soft, Easily worked.	Hair dyes, Pottery lead glazes, insecticides, Lead piping.	Hypotention, Miscarriages, Renal impairment, Brain injury, Abdominal pain.	*Asparagopsis sps* *Chlorella vulgaris* *Chlorella vulgaris* *Chlorella kessleri*		[158] [150] [144] [159] [159]

In Hg and Cd-treated cells, a noticeable degradation of cell organelles was observed. The starch grain accumulation in *P. typicum,* which was treated with heavy metal, served as an energy store for the organelles of the cell, such as the pyrenoid, mitochondria, and chloroplast, and matched with the report given by Wong and coworkers regarding *Chlorella fusca* [129, 142, 160]. They observed that the green algae species *Chlorococcum sp, Fischerella sp, vulgaris, Scenedesmus sp,* and *Chlorella vulgaris var.* removed the most mercury with 97%, 96 %, 94%, and 92%, respectively. *Tolypothirx tenuis, Phormidium molle, Stigonema sp., and Lyngbya spiralis* are blue-green algae species that removed the most mercury with 96%, 94%, and 93%, respectively [142].

Transgenic approaches are established to develop the heavy metal specification and binding capability of microalgae. In one such approach, the eukaryotic microalga *Chlorella* sp. DT was found to facilitate the decline of Hg_2 to volatile elemental Hg° when altered with the *Bacillus megaterium* strain MB1 merA gene codes mercuric reductase (MerA). The merA gene was functionally expressed and inserted into the genetic composition of transgenic strains to enable Hg2 removal [161]. *Chlorella fusca var. fusca, Selenastrum minutum, diatom Navicula pellicosa, and thermophilic alga Galdiera sulphuraria* demonstrated the majority of Hg0 volatilization within 20 min to a few hours. Thiol chelation and bio-methylation to methylmercury are two additional possible mechanisms for Hg(II) removal [78]. Because of its high efficiency, the combination of activated sludge and membrane filtration in membrane bioreactor systems for wastewater treatment has received much interest. Microalgae dynamic membranes made from *C. vulgaris* powder were used in a dynamic membrane bioreactor to bioremediate Hg^{2+} from synthetic dentistry effluent, with the aid of increased elimination yield, reduced fouling, and lower membrane recovery costs (Table 4) [24, 162].

5.2. Zinc removal

Cyanobacteria *Microcystis subspicatus* had the maximum zinc uptake of 999.50 mg metal ion/ g biomass by microalgae. Macro algae *Bifurcaria bifurcata, Laminaria hyperborean, Sargassum muticum*, and *Fucus spiralis* were able to remove not only Pb and Cd but also Zn from the solutions. [163] found that another microalgal species, *Scenedesmus obliquus*, had similar effectiveness in removing Zn. At pH 6, this freshwater microalga was capable of eliminating 112 mg Zn/g biomass content (78%). *Desmodesmus pleiomorphus* was tested in a study conducted by [163]. It was able to extract 83.1 mg Zn/g of biomass. Low Zn concentrations were also found to be favorable to the growth of *D. pleimorphus. Chlorella vulgaris,* which has been extensively studied, can also remove specific levels of Zn along with Cu.

Several investigations found that the best elimination of Zn occurred at pH 3.5 and 6.5. The maximum adsorption and intracellular uptake occurred with this microalga at 35°C and 25°C, respectively [130].

Table 4. Metrics for heavy metal removal for various algae genera.

Metal	Algal Genus / Species	Uptake Capacity (mg g^{-1} dry biomass)	Removal Efficiency (%)	Initial Concentration & Contact Time	Toxicity Threshold / Notes	Refs.
Mercury (Hg^{2+})	*Phormidium ambiguum*	–	70%	5 mg/mL, 0.5 h	Highly sensitive at low concentrations	[24,78,129,130,142 160,161,162]
	Pseudochlorococcum typicum	–	70%	5 mg/mL, 0.5 h	Cell degradation above 20 mg/mL	
	Scenedesmus quadricauda	–	70%	5 mg/mL, 0.5 h	Sensitive at 5–10 mg/mL	
	Spirogyra hyalina	39.212 mg/g	–	40 mg/L, 120 min	–	
	Spirulina subspicatus	1.4 mg/g	60%	–	Ineffective compared to others	
	Chlorella fusca, Scenedesmus sp., Fischerella sp.	–	92–97%	–	–	
	Transgenic *Chlorella sp.* (merA)	–	Volatilizes Hg^0	–	Enhanced removal via reductase	
Zinc (Zn^{2+})	*Microcystis subspicatus*	999.5 mg/g	–	–	Extremely high uptake	
	Scenedesmus obliquus	112 mg/g	78%	pH 6	–	[85, 130, 163]
	Desmodesmus pleiomorphus	83.1 mg/g	–	–	Low Zn enhances growth	
	Chlorella vulgaris	–	–	Best removal at pH 3.5–6.5	Temp-dependent uptake (25–35°C)	
	Multiple genera (live & dead biomass)	–	0.3–360%*	pH 3–7	Broad variability across 20+ species	
Copper (Cu^{2+})	*Scenedesmus sp.*	–	–	0.5 mg/L inhibits growth	Growth inhibition threshold	[93, 164-168]
	Cladophora glomerata, Oedogonium rivulare	–	Significant	–	–	
	Ulva reticulata, Padina gymnospora	–	Accumulation increases with concentration/time	–	Brown algae > green algae in uptake	
	Mixed algal consortia	–	71–85%	Autoclaved effluent	Dead biomass often more effective	
	Tetraselmis marina AC16-MESO	–	42.9–92%	1–5 mg/L	Stimulatory at low Cu (0.1 mg/L)	
Arsenic (As III/V)	*Phaeodactylum tricornutum*	–	–	1 µM	35% growth inhibition	[23,26,29,169-173]

	Chlorella sp.	–	–	Up to 200 mg/L As(V)	High tolerance	
	Nannochloropsis sp.	–	–	Up to 100 µM As(III)	Decline above 500 µM	
	Hydrodictyon reticulatum, *Phormidium, Pithophora*	–	–	–	Accumulates up to 760 µg/g	
	Chlorella minutissima, *Scenedesmus sp.*	–	Removal up to 161 µg/g	Up to 500 mg/L	Reduced proteins & chlorophyll	
Cadmium (Cd^{2+})	Transgenic *Chlamydomonas reinhardtii*	–	Increased uptake	–	Improved tolerance via gene modification	[48,99, 84, 174-176]
	Parachlorella hussii, *P. kessleri*, *C. luteoviridis*	–	High tolerance & uptake	–	–	
	Chlorella sp.	–	Good uptake	–	–	
	Ankistrodesmus sp., *Scenedesmus sp.*	–	Variable	–	*Scenedesmus* more tolerant	
	Desmodesmus pleiomorphus	–	Reliable removal	pH-dependent	–	
	Haloferax (Archaea)	–	High	0.5–1 mM	Extreme tolerance	
	Parachlorella sp.	–	–	–	Functional groups improve sorption	
Chromium (Cr VI)	*Phaeodactylum tricornutum*, *Navicula pelliculosa*	–	–	–	Cr(VI) biosorption	[54, 56, 177]
	Chlorella sorokiniana	–	–	100 ppm, 3 days	High tolerance	
	Rhizogonium hookeri	–	–	–	Porous surface aids adsorption	
	Navicula subminiscula	–	Up to 98%	20 mg/L	High tolerance	

Isochrysis galbana, Planothidium lanceolatum, and *Scenedesmus subspicatus* in their live forms at pH of 5, 7, and 6 can uptake Zn at 0.3%, 118.66%, and 72.06%, respectively. Other species, including *Arthrospira (Spirulina) platensis* (33.21%), *Aulosira fertilissima* (19.15%), *Chlorella homosphaera* (15.6%), *Cyclotella cryptica* (242.9%), *Desmodesmus pleiomorphus* (360.2%), *Euglena gracilis* (7.5%), *Hydrodictyon reticulatum* (3.7%), *Phaeodactylum tricornutum* (14.52%), *Phormidium spp*. (9.4%), *Pithophora odeogonia* (8.98%), *Porphyridium purpureum* (2.01%), *Scenedesmus obliquus*(6.67%), *Scenedesmus quadricauda* (5.03%), *Spirogyra insignis* (21.1%), *Spirogyra neglecta (*31.51%), *Spirulina platensis (*7.36%), *Spirulina spp*.(0.17%), and *Stigeoclonium tenuecan* (0.77%) take up the metal in their non-living form of biomass at their respective pH [85].

5.3. Copper removal

Scenedesmus was cultivated in various copper concentrations. Cu had a value of 0.5 mg/L that inhibited *Scenedesmus* growth. *Cladophora glomerata* and *Oedogonium rivulare* also exhibited significant copper decontamination [164]. The brown alga *P. gymnospora* was studied for its ability to absorb two metals, Cu and Zn, and Cu uptake by *Ulva reticulata* has been examined. Both macroalgae absorb metals in proportion to their concentration in the growth medium ($p < 0.05$); the greater the heavy metal exposure concentration in the growth medium, the larger the metal accumulation. It was also found that the exposure period had a significant impact on the deposition. Brown macroalgae have a high accumulation capacity when compared to green algae. This was observed by the occurrence of polysaccharides with a higher affinity for cations in their cell wall material, along with the occurrence of physodes – bodies comprising phenolic compounds which gather great quantities of heavy metals in their cell wall material [165-168].

Chan et al., 2014 conducted a few laboratory-scale experiments on three microalgal species, viz, *Chlorella vulgaris* (green algae), *Spirulina maxima* (blue-green algae), and a certain growing algal sample, in plant wastewater. They found it contained *Chlorella* sp. (common strain), *Synechocystis* sp. (dominant strain), and *Scenedesmus* sp. In the method implemented, one of the effluents was autoclaved while the other was untreated. The results revealed that the removal efficiency of the algal species used in the autoclaved trial was better than the untreated trial. According to Simeonova et al. (2008), dead cells ingest metal ions in the same or better proportion as living cells, which could explain why the autoclaved trial achieved similar or better clearance efficiencies in less time. In the autoclaved trial, the Cu removal efficiency ranged from 70-85%, and there was a rapid decline in its concentration. Along with the single strains, they used mixed strains of algal species to determine the synergistic effect on metal removal. According to their perceptions, mixed strains were much more efficient at metal removal with the following percentages: MIX-71.7, CV/MIX−82.8, CV/SM/MIX−76.6, SM/MIX−84.4, and CV/SM-79.5 [138]. For Cu^{2+}, the removal percentage by *Tetraselmis marina* AC16-MESO was 42.9% at 1.0 mg L−1 and 92% at 5.0 mg L−1. Cu^{2+} was shown to enrich the progress rate of microalgae *Scenedesmus obliquus* and *Chlorella pyrenoidosa*. Cu^{2+} had a favorable effect on the development of *Tetraselmis marina* AC16-MESO at a concentration of 0.1 mg L1, resulting in a 21% growth in cell density during the 72 hours associated with the control [93].

5.4. Arsenic removal

Microalgae are a potential tool that can reduce toxicity in As (III) through oxidation of inorganic arsenic, and also complex formation. As(V) is biotransformed into arsenolipids/arsenosugars or methylated arsenic species, reduced, adsorbed on cells, and excreted from cells into less toxic forms [139]. Cellular metabolism of arsenic converts inorganic forms of As (V) and As (III) into the less toxic organic forms of MMA and DMA [170]. Freshwater microalgae and marine water microalgae differ in their tolerance to arsenic, which affects the development rate and bioaccumulation ability in the presence of arsenic. Observations of marine algae *Phaeodactylum tricornutum* in its bioaccumulation experienced concentrated inhibition of 35% at 1.0µm arsenic [171]. Yan and Zhao, 2025 found a difference in the tolerance capacity between *Chlorella Sp* and *Monoraphidium arcuatum,* with a more and less tolerance capacity to arsenic, respectively [172]. *Chlorella vulgaris* shows a high tolerance to As (V) of up to 200 mg/L, as observed by Jiang and co-workers [173]. Upadhyay and co-workers observed that *Nannochloropsis* species grew well in As (III) concentrations up to 100µm, but biomass considerably decreased with an increase in concentration up to 500µm [29].

Singh and colleagues reported that *Hydrodictyon reticulatum*, Diatoms, *Pithophora* species, *Phormidium* species, and *Oscillatoria* species were found in an As-polluted area, suggesting possible bioremediation of arsenic by the accretion of arsenic up to 760 µg/g [26]. Arora and co-workers observed that *Chlorella minutissima and Scenedesmus* species can bear up to 500 mg/L arsenic, with elimination up to 161 µg/g. Oxidative stress induced by arsenic had different effects on different microalgae, with a reduction in protein and chlorophyll content in the *C. minutissimum* and *Scenedesmus* species and cell size reduction in *C. minutissima* [23].

5.5. Cadmium removal

Strategies, such as genetic engineering, microalgae immobilization, bio pellets, and bio-immobilization, are used for cadmium biosorption. To increase heavy metal tolerance, gene manipulation was done in the protein of *Chlamydomonas reinhardtii,* showing a great increase in Cd^{2+} tolerance and uptake [99]. Few microalgal species, like *Parachlorella hussi, Parachlorella kessleri, and Chlorella luteoviridis,* showed great metal accumulation and tolerance as well [174]. *Chlorella* sp. had a good capacity for Cd (III) biosorption. [48] and his coworkers observed three strains of microalgae, *Ankistrodesmus* sp, *Chlorella* sp, and *Scenedesmus* sp., for their Cd tolerance and concluded that *Ankistrodesmus* Sp showed less tolerance compared to the other two. Monteiro and colleagues investigated two strains of *Desmodesmus pleomorphic* for their potential in removing Cd at different pH levels; both strains proved to be reliable tools to remove Cd [92]. Das and coworkers gave insights on cadmium removal using *Haloarchaea,* which showed efficient metabolism in all extreme conditions. They were tolerant to cadmium at high concentrations. *Haloferax* strain BBKZ removed Cd by accumulation and showed great heavy metal resistance at a concentration of 0.5mM-1mM [175]. Work by Dirbaz and Roosta showed that the acidic functional groups of carboxylic acid, amide functional groups -OH, -NH, C=O, and alcohol functional groups C-O possessed by *Parachlorella* sp. were a better biosorbent for cadmium than other species, such as *Nannochloropsis, Scenedesmus* sp., and *Spirulina* sp. [176].

5.6. Chromium removal

Microalgae adopt several mechanisms in the process of decline and elimination of Cr (VI). Biosorption of Cr (VI) was one such mechanism adopted by the *Phaeodactylum Tricornutum* and *Navicula Pelliculosa* microalgal strains [54]. Reduction of Cr (VI) to Cr (III) also reduced the toxicity of hexavalent chromium. *Chlorella sorokiniana*, a good biosorbent of Cr (VI), showed efficient tolerance on exposure, i.e., 100 ppm Cr (VI) for three days [56]. Freshwater microalgae *Rhizogonium Hookeri* is also a good adsorbent of Cr (VI) due to the porous nature of its surface. Cherifi and coworkers reported that *Navicula subminiscula* species removed heavy metal up to 98% at a concentration of 20 mg/L Cr (VI) and had a high tolerance capacity [177].

In summary, microalgae use a variety of processes to eliminate harmful heavy metals: extracellular biosorption, enzymatic reduction, compartmentalization, intracellular bioaccumulation, bio-methylation, and volatilization. The investigation of mechanisms involved in the phycoremediation of heavy metals offers suggestions to increase efficiency and selectivity, as well as lowering processing costs, even though the process is still at a lab scale.

6. Recovery process

As valuable industrial products with financial benefits, these metals are attractive for recovery from wastewater. However, few studies have been carried out on the recovery of these precious metals from the soil, as most of the remediation techniques, such as chemical precipitation, membrane filtration, adsorption, and ion exchange, focus on removing these precious metals [178]. Material cycling and safe post-treatment involve the extraction and recycling of metals from metal-loaded biomass used in the bioaccumulation and phytoremediation processes that treat industrial wastewater. By using the proper eluents to desorb, the metals adsorbed on the surface of biosorbents can be retrieved.

However, metals that have interacted with the intracellular components of microbial and plant cells can be recovered by leaching using chemical or biological reagents, or through carefully controlled heat processing [179].

6.1. Biosorbents

Current research on renewing biosorbents and recovering metals from metal-loaded biosorbents uses chemicals. Heavy metal cations can be safely removed from metal-containing biosorbents by applying diluted acids (such as HCl or HNO3).

The polysaccharides on the biomass surface that served as metal binding sites were hydrolyzed, likely causing HCl to lose some of its metal adsorption capacity in the regenerated algal biosorbents, despite the fact that HCl has a considerable capacity for metal desorption [82].

Because they have a high capacity to eliminate metal cations from biosorbents and function as electrolytes in the electrolytic method for retrieving the metal, diluted acid solutions comprising inorganic salts, like CaCl2 and NH4Cl, are also appropriate for this role. Electrolysis can be used to remove the metal ions from the eluate produced during the desorption process. If the amount of metal cations in the eluate is restricted, the use of rotating cathode cells would be helpful in retrieving the metals. There are a variety of different adsorption-desorption cycles that can be used with commercial biosorbents. The silica gel-immobilized AlgaSORBTM could be utilized for more than 100 adsorption-desorption cycles. When HCl or HNO3 is employed as the eluent, BioFix biosorbent is suitable for more than 120 cycles [180].

But after five cycles, the regenerated biosorbent's Cd(II) adsorption value decreased to 53.4% of the initial value [181]. Adsorbed Cd(II) was easily desorbable with distilled water on pyrolyzed coffee debris bound with clay, yielding a recovery of 88.0-91.9%. Through fermentation, composting, and burning, the consumed biosorbents were transformed into energy fuels and thermal energy.

6.2. Leaching

The elimination of heavy metals from residual biomass in the remediation of metal-contaminated industrial waste waters can be accomplished by chemical removal or bioleaching, which has been extensively studied for its application in contaminated wastewater. Similar to sediment leaching, bioleaching uses bacteria that oxidize iron and sulfur, such as A. thiooxidans and A. ferroxidans, to dissolve heavy metals from metal-laden biomass.

Chelating agents, inorganic salts, and inorganic acids can be used in the chemical extraction process as extracting agents. Various organic acids have been found to be more efficient than other removing agents in the elimination of heavy metals from sewage sludge between pH 1.5 and 2; however, post-treatment is necessary to neutralize the treated sludge. Meanwhile, organic acids, particularly citric acid, have been revealed to be effective in recovering heavy metals below mild acidic environments (pH 3–4). In order to minimize environmental impact and maximize material recycling, the leachate metals have to be recovered as well. After leaching, metal-contaminated biomass can be fermented for the production of energy fuel, thermally treated, or composted [179, 182].

6.3. Thermal treatment

Phytoremediation and bioaccumulation of metal-contaminated biomass can be processed thermally by combustion at high temperatures under ambient [aerobic] conditions and pyrolysis at moderate temperatures under anaerobic conditions. Existing solid waste from municipal incinerators can burn biomass that has been enhanced with metals. In the combustion process, the biomass is decomposed by organic matter, and the metals are released as oxides. The released metals are either discharged into the flue gas or entrapped in ash. Modern flue gas-cleaning technologies, such as dust scrubbers and flue gas desulfurization equipment, would guarantee the successful capture of metal-containing dusts, with the exception of volatile, non-condensable elements such as mercury. Metal-containing substances from biomass can also be incinerated in furnaces that employ rotary kiln technology and can handle various substances to recover metals. Combustion produces thermal energy, which can be transformed into heat and electricity. A possible drawback of burning metal-loaded biomass is the generation of secondary pollutants, such as ash-concentrating non-volatilized heavy metals and worn dust filters from flue gas-cleaning equipment that have accumulated volatilized metals. Nonvolatile heavy metals in the unique biomass will be concentrated in the char fraction when biomass is pyrolyzed under entirely hermetic and oxygen-free circumstances.

The behaviour of heavy metals in thermal processes depends on a number of factors, including the metals' characteristics, the physicochemical properties such as temperature and oxidation-reduction status, and the presence of reactive chemicals in the reactor. [155] stated that of the metals present in contaminated wastewater, Cr and Ni are non-volatile species, As, Cd, and Hg are volatile, while Cu, Pb, Sn, and Zn are intermediate. Since nonvolatile salts like oxides and sulfates are easier to form in oxidative conditions, volatile gaseous metal compounds like hydroxides, chlorides, and sulfides may remain in reductive conditions. Trace metals are typically more volatile in reductive conditions during pyrolysis than in an oxidative atmosphere during combustion [183]. The original biomass's moisture, chlorine, and sulfur contents may have impacted the volatility of the HMs [179, 184].

In order to extract and recover metals from metal-loaded biomass, the electrokinetic technique and supercritical fluid extraction could be used. The electrokinetic method relies on electrophoresis, electro-osmosis, and electrolysis processes that occur when the metal-containing substrate is charged electrically with a direct current using one or more electrode arrays. Essentially a liquid extraction method, supercritical fluid extraction uses compressed fluids—typically either liquids or gases—under supercritical conditions in place of conventional solvents.

7. Metallothionein (MT) and relevant gene studies

Metal ions at low concentrations are involved in several cellular functions, such as signaling, energy transfer, and acid-based chemistry or redox potential, while they are detrimental at higher doses.

Consequently, the cells develop metal-resistance mechanisms. It appears in two mechanisms of fundamental metal resistance: intracellular chelation and metal membrane transport. Phyto-chelatins are intracellular chelators that are synthesized in response to metal stress, but MT is formed through assorted spurs [85, 121]. MTs are predominant in all existing life forms and perform a critical part in providing vital metals to the cell, including the transport of noxious metals into other organelles, cell cycle modulation, metal homeostasis, and regulation of various kinds of stress, including metal stress. It has also been proposed that they act as defending cells from oxidative stress and as free radical scavengers (Figure 3) [117, 185].

MTs are a class of low-molecular-weight, cysteine-rich proteins that play an essential role in the detoxification and homeostasis of heavy metals in living organisms, including microalgae. MTs are the small molecular weight proteins that are named due to the presence of a number of sulfhydryl groups and associated metals [186]. More precisely, MTs are proteins rich in cysteine, and they are categorized by cysteines structured in motifs. Their capability to interact with metals is mainly due to the presence of cysteine thiol groups, but it can also be attributed to the existence of histidines and a nitrogen lateral chain [187, 188]. These proteins are characterized by their high affinity for metal ions, particularly cadmium (Cd), zinc (Zn), copper (Cu), lead (Pb), mercury (Hg), and arsenic (As).

The presence of numerous cysteine residues allows MTs to form stable thiolate bonds with these metal ions, facilitating their sequestration and reducing their toxicity within biological systems [189].

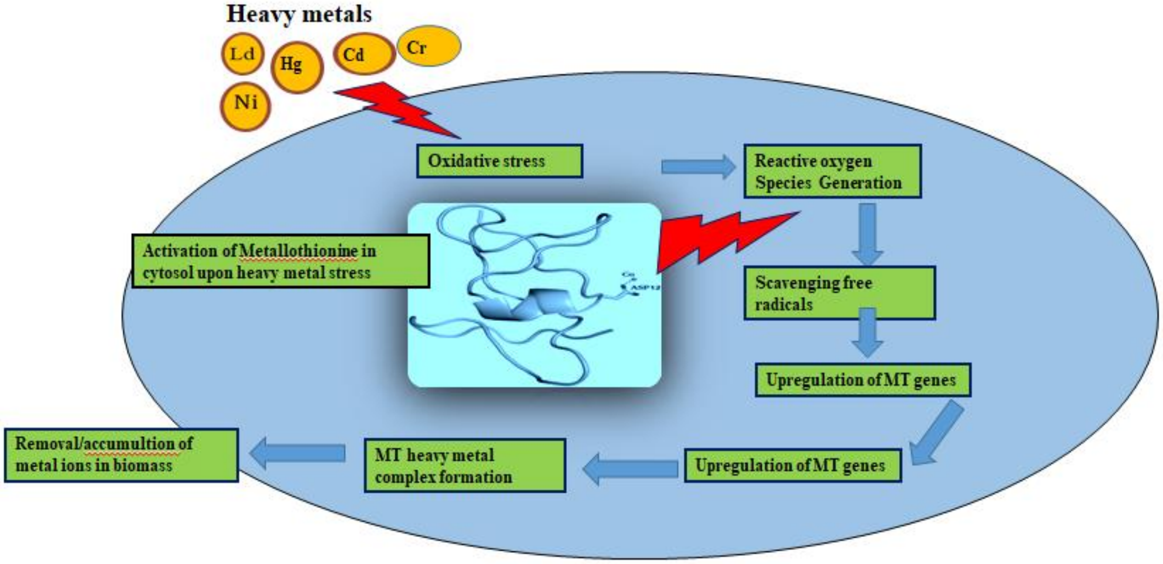


Fig. 3. Probable echanism of metallothionine mechanism in bioremediation of heavy metals.

They have minimal or even no content of aromatic amino acids and can inhibit metals. They are prevalent, particularly in Eukaryotes, although they arise in a small number of bacteria, including Cyanobacteria [185]. Structurally, metallothioneins are unique due to their lack of a well-defined secondary or tertiary structure in their metal-free state. However, upon binding metal ions, they adopt a compact, functional conformation

The cysteine residues in MTs are organized in specific motifs. These motifs are responsible for coordinating metal ions in a dynamic, reversible manner. The proteins typically fold into two main domains: the α-domain, which binds divalent metals such as Zn(II) and Cd(II), and the β-domain, which has a higher affinity for monovalent metals such as

Cu(I) [190]. Functionally, metallothioneins contribute to heavy metal homeostasis by regulating essential metal concentrations in cells, mitigating oxidative stress, and providing cellular protection from metal-induced toxicity. Their metal-binding capacity allows them to act as metal reservoirs, ensuring that cells maintain optimal levels of essential metals like Zn and Cu while preventing the toxic accumulation of heavy metals [191]. Additionally, metallothioneins exhibit antioxidant properties by neutralizing reactive oxygen species (ROS) generated due to metal stress, thereby protecting proteins, lipids, and nucleic acids from oxidative damage.

In plants, MTs are involved in various metabolic activities, including embryogenesis, root development, ripening of fruit, senescence, and pollen germination. Transcript levels of MTs are observed to vary under varied biotic and abiotic stress conditions [192]. MTs, also known as metal-binding proteins, are involved in metal stress tolerance, metal regulation, and protection of the cell from metal toxicity. MT expression levels indicate the heavy metal stress condition of the environment and, hence, serve as a biomarker. During a chronic metal toxicity study, MT acted as a multi-functional stress protein [193]. MT are localized in the cytosol and predicted in *Symbiodinium microadriaticum* (dinoflagellates; GenBank accession number OLP85454) and *T. pseudonana* (diatom; *XP_002296843*). Genetic modifications in algae for heavy metal removal and adsorption were studied. The *C. reinhardtii* plastid genome was cloned with *Festuca rubra* MT genes, resulting in higher cadmium uptake. MTs have applications as biosensors and in biomedical science as drug transporters and biomarkers due to their high affinity for heavy metals [117].

Homo sapiens metallothionein is coded with 17 genetic codes on chromosome 16q13. They are made up of 61-68 amino acid residues, with cysteine accounting for 25-30% of all amino acids and no aromatic amino acids. MTs are categorized into four isoforms. The MT-2, MT-3, and MT-4 genes are all encoded by a single gene, but the MT-1 gene has many subtypes (MT-1A,-B,-E,-F,-G,-H,-M,-X) [185, 186, 188, 193].

The MTs exist in primary, secondary, and tertiary structures. The primary contains conserved cysteine motifs. The Cys Motifs are xxCxx, CxC, xCCx, CxCxC, CxCC, CCxC, CCC, CxCxCxC, and CCxCC. There are a total of nine different motifs, where x is any other amino acid. Without metals, the apoprotein employs a compressed orientation with some cysteines on the peripheral plane rather than a true secondary structure. This confirmation may inhibit cysteine oxidation while also allowing peptide metal interaction. At acidic pH, the apoprotein lacks this pseudo-organization and becomes much looser. When MTs are bound to metals, they form a tertiary structure. Divalent metals (Me (II)), such as zinc and cadmium, can bind 4 Cys (tetragonal coordination), whereas 2–3 Cys can be bound by monovalent metals (Me(I)), such as Cu(I) or Ag (diagonal or trigonal coordination). Because of this, MTs certainly bind to Me (I) instead of Me (II), and the type and quantity of bound metals dictate the 3D structure, which can take many different forms. There are three molecular types of MTs in cells: (i) Thionins (TOs), which are poorly reacting oxidized forms; (ii) Reduced forms of thioneins (Ts-) that have the ability to attach to reactive chemical species, such as reactive metal species (RMS), reactive nitrogen species (RNS), and reactive oxygen species (ROS); and (iii) MTs attach to a variety of proteins and are extremely active metalated versions [185].

8. Computational study: a key for phycoremediation mechanism insights

Microalgae exhibit varied mechanisms in reducing heavy metal noxiousness, such as adsorption sites and protein interactions. MTs are a family of cysteine-rich, low molecular weight proteins involved in dual functions, i.e., heavy metal removal and antioxidants. MTs have the ability to interact with the thiol group of cysteine residues [154]. Structurally, MTs contain multiple cysteine (-SH) residues, forming thiolate bonds with metal ions. The protein folds into two domains (α and β), creating a metal-binding core [189]. These thiol clusters effectively bind heavy metals, such as cadmium, mercury, and lead, reducing their toxicity and facilitating safe sequestration [194]. Functionally, metallothioneins contribute to metal sequestration, oxidative stress protection, and metal transport and storage. By binding toxic metal ions, MTs prevent cellular damage while simultaneously regulating essential metal ion homeostasis. They act as antioxidants by scavenging reactive oxygen species (ROS) generated by metal-induced oxidative stress [117]. MTs also facilitate intracellular metal transport, ensuring that necessary metals, such as zinc and copper, are available for metabolic processes while excess toxic metals are neutralized.

In vascular plants, MT is a prerequisite for metal ion homeostasis and sulphur metabolism. In plants, metallothionein expression was higher during abiotic stress conditions and involved in scavenging reactive oxygen species generated during oxidative stress [195-197]. Microalgae use metallothioneins for metal detoxification through several mechanisms. Biosorption enables MTs to adsorb metal ions onto the cell surface, preventing intracellular toxicity. Bioaccumulation allows MTs to transport metals into vacuoles for long-term storage and sequestration. Additionally, MTs regulate antioxidant enzyme activity, minimizing oxidative stress resulting from metal exposure. They also play a role in redistributing essential metals, like zinc and copper, while binding excess toxic metals. Studies on *Chlorella*, *Spirulina*, and *Phormidium* have shown that MT overexpression enhances metal tolerance, reinforcing the suitability of these microalgae for bioremediation applications [198, 199]. In algae, the metallothionein correlated sequence has been reported to be up-regulated in copper-induced stress in *Fucus vesiculosus.* The transcript is reported to interact with copper and cadmium [200].

Microalgae unveil vital mechanisms for the exclusion of free metal ions from waters, thereby both depolluting and bio-remediating. Metallothionein peptides are involved in a hasty reaction of the cell to impulsive and persistent heavy metal stress; it is an energy reliant transference to the cell vacuole in addition to mechanisms such as reactive oxygen species that converse tolerance to microalgae species [201,202]. Upregulation of MT leads to the accumulation of misfolded forms, eventually forming unstable proteins; hence, the overexpression of MT does not signify metal uptake, as it actually decreases the metal uptake. Synergic overexpression of certain soluble proteins and MT has resulted in higher metal uptake. Bioengineered transgenic *C. reinhardtii* infused with chicken MT expressed fluorescent protein to screen the accumulation of heavy metal, revealing the biosensor application [122].

The binding efficiency, adsorption potential, and hydrophobic and hydrophilic bonding of various microalgae exhibit varied sorption sites and mechanisms towards different heavy metals. In-depth studies unravel the molecular mechanism of the adsorption of the microalgae. Here, an attempt is made to understand the binding affinity of three microalgae metallothionein proteins to different heavy metals through molecular modelling and a computational study with MT as the target site (Figure 4). This may help portray the bioremediation efficacy through *in silico* models with simulation studies to generate large-scale bioremediation models in industries.

The study focused on three microalgal species, *Phormidium tenue*, *Chlorella sorokiniana*, and *Spirulina platensis,* that were selected for their heavy metal tolerance and bioaccumulation potential. *Phormidium tenue* is a cyanobacterium known for its high metal-binding capacity, facilitated by extracellular polymeric substances (EPS). It produces exopolysaccharides that enhance heavy metal sequestration [88] and is tolerant to arsenic, cadmium, and lead. *Chlorella sorokiniana* is a fast-growing green microalga with high bioaccumulation potential, particularly for cadmium and chromium It has been extensively used in wastewater treatment due to its ability to accumulate and transform toxic metals [203]. *Spirulina platensis* is a filamentous cyanobacterium recognized for its high biomass productivity and ability to accumulate zinc, copper, and lead. It has strong antioxidant properties that mitigate oxidative stress induced by heavy metals, making it suitable for large-scale detoxification applications [81].

8.1. Methodology

8.1.1.Protein and ligand processing

Metallothionein protein sequences of *Spirulina platensis*, *Phormidium tenue,* and *Chlorella sorokiniana* were retrieved from the UniProt database (Table 5) (The UniProt Consortium).

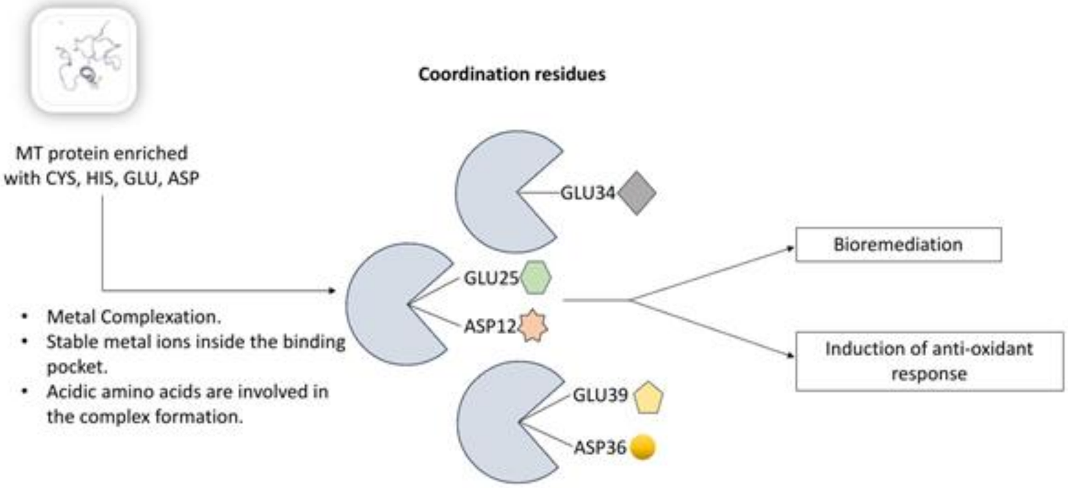


Fig. 4. Systematic epresentation of heavy metal bioremediation by *in silico* approach.

Table 5. Details of Templates used for Modelling MT Proteins

Organism	UniProt Accession No	Length	Template PDB ID	Sequence Identity (%)	Ramachandran Plot Favoured region (%)	Allowed Region (%)
S. platensis	D4ZYS3	55	1JJD	69.81	87.5	100
P. tenue	A0A1U7J8X1	56	1JJD	58.18	88.0	100
C. sorokiniana	A0A2P6TKK5	56	1DMD	38.46	63.0	81.5
			1IJ5M	53.57		

The MT sequences of *S. platensis* and *P. tenue* were modelled using the SWISS-MODEL [204], and the sequence of *C. sorokiniana* was modelled using Modeller10.1 [205].

The modelled MT sequences were energy minimized using GROMACS 2020.1 with the OPLS forcefield [206]. The modelled structures were verified by a Ramachandran plot [207]. The following metal ions were retrieved from PubChem: Hg^{2+}, Cu^{2+}, Cd^{2+}, Pb^{2+}, and Ni^{2+} for *S. platensis;* As^{3+}, Co^{2+}, and Fe^{3+} for *P. tenue;* and Cd^{2+}, Zn^{2+}, and Cu^{2+} for *C. sorokiniana* [208].

8.1.2. Molecular docking

Molecular modeling and docking techniques were used to analyze the interactions between MT proteins and heavy metals. The AutoDock 4.2 package was used for docking metal ions into MT proteins [209]. The MT proteins were prepared by adding hydrogen atoms. Partial charges were calculated using the Kollman method. Grid parameter files were generated using AutoGrid4. The grid box was set up to cover the metal ion binding site. The grid spacing was set to 0.375Å. Docking simulations were conducted using a grid box encompassing the entire metallothionein active site to allow for optimal exploration of the binding site. Docking simulations were carried out using the Lamarckian genetic algorithm. Docking parameter files were generated using AutoDock4 with default settings. The bound metal ion and coordinating residues were visualized in PyMol (The PyMOL Molecular Graphics System, Version 2.0).

8.2. Results

This study evaluated the interaction of microalgal metallothionein proteins from *Phormidium tenue*, *Chlorella sorokiniana,* and *Spirulina platensis,* with a panel of ecotoxicologically relevant heavy metal ions using molecular docking.

For each MT, between three and five metal ions were docked (total n = 11 protein–metal combinations), and the best-ranked pose in the dominant cluster was analyzed in terms of binding energy (kcal/mol), size of the lowest-energy cluster, coordinating residues, and the presence of steric clashes.

The binding energy of MT ranged between –2.86 to –1.41 kcal/mol for S. platensis, -16.67 to –3.24 kcal/mol for *P. tenue,* and –5.90 to –3.21 kcal/mol for *C. Sorokiniana* (Table 6).

8.2.1 Overall binding energy trends

The MT from P. tenue showed the strongest overall metal-binding propensity, with docking-derived binding energies ranging from −16.67 to −3.24 kcal/mol, followed by *C. sorokiniana* (−5.90 to −3.21 kcal/mol) and *S. platensis* (−2.86 to −1.41 kcal/mol). The strongest binding was observed in *P. tenue*, indicating its superior potential for heavy metal detoxification.

Higher binding energy values (closer to zero) indicate weaker interactions, making metals more bioavailable for sequestration and detoxification. These results align with previous metallothionein studies, where lower binding energies correlate with enhanced metal-binding capabilities [194, 210].

The binding site of the MT proteins was enriched with CYS, HIS, GLU, and ASP residues, which favors metal ion complexation. The binding site residues were conserved in all three MT sequences (Figure 5). These values indicate that P. tenue MTs form particularly stable complexes with Fe, Co, and As, while the MTs of *C. sorokiniana* and *S. platensis* exhibit moderate but consistent affinities across their respective metal panels. In all three species, the lowest-energy poses were also represented by the largest clusters of docking conformations, supporting the robustness of the predicted binding modes.

8.2.2 Metal coordination by Spirulina platensis MT

Docking of Hg^{2+}, Cu^{2+}, Pb^{2+}, Cd^{2+}, and Ni^{2+} to S. platensis MT yielded binding energies between −2.86 and −1.41 kcal/mol, with Hg^{2+} and Cu^{2+} forming the most favorable complexes in this panel (Figure 6).

The binding pocket is enriched in Cys, His, Glu, and Asp residues, and a conserved Glu34 residue repeatedly participates in coordination across several metals, acting as a key anchoring site within the MT scaffold. Visual inspection of the complexes showed predominantly compact, well-packed geometries with minimal steric clashes, suggesting that S. platensis MT can support multi-metal binding while maintaining structural compatibility with different ionic radii and coordination preferences.

8.2.3 Metal coordination by Phormidium tenue MT

Among all systems analyzed, *P. tenue* MT displayed the most negative binding energies, with Fe^{3+}, Co^{2+}, and As^{3+} complexes spanning −16.67 to −3.24 kcal/mol. Fe^{3+} and Co^{2+} interacted strongly with Asp12 and neighboring acidic residues, forming dense coordination shells consistent with high-affinity metal sequestration (Figure 7).

In contrast, As^{3+} binding was accompanied by steric clashes with Glu25, indicating that although the MT can interact with arsenic, local geometric strain may reduce complex stability relative to Fe and Co.

These patterns support *P. tenue* MT as a particularly promising candidate for Fe/Co-rich effluents, while also highlighting structural constraints that may limit optimal arsenic coordination.

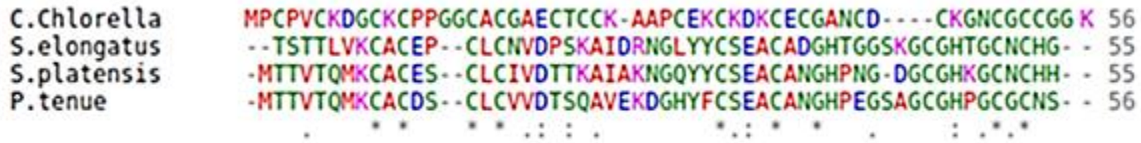
8.2.4 Metal coordination by Chlorella sorokiniana MT

For *C. sorokiniana,* docking was performed with Cd^{2+}, Zn^{2+}, and Cu^{2+}, yielding binding energies from −5.90 to −3.21 kcal/mol, intermediate between the strong affinities seen for P. tenue and the milder affinities of *S. platensis.* Zn^{2+} and Cu^{2+} formed stable complexes coordinated primarily by

Asp36, with additional support from neighboring acidic and polar residues, while Cd^{2+} binding introduced steric clashes with Glu39, suggesting a less optimal fit of Cd^{2+} in the *C. sorokiniana* MT pocket (Figure 8). These results are consistent with experimental reports of efficient Zn and Cu uptake by *Chlorella* species, and point to a mechanistic basis for differential metal preferences within the same MT scaffold.

Table 6. Binding Energy and Coordination Residues of Docked Complexes

Organism	Metal ion	Lowest Binding Energy (kcal/mol)	Coordination residue	Distance from residue to metal ions (Å)
S. platensis	Cd	-2.05	GLU34	Cd-O: 2.06 Cd-O: 2.01
	Hg	-2.43	GLU34	Hg-O: 2.06
	Cu	-2.41	GLU34	Cu-O: 2.06
	Pb	-1.41	GLU34	Pb-O: 2.85
	Ni	-2.86	GLU34	Ni-O: 1.87 Ni-O: 2.5
P. tenue	As	-3.73	GLU25	Steric clashes
	Co	-3.24	ASP12	Co-O: 1.95
	Fe	-16.67	ASP12	Fe-O: 1.17
C. sorokiniana	Cd	-3.53	GLU39	Steric clashes
	Zn	-3.21	ASP36	Zn-O: 1.73
	Cu	-5.90	ASP36	Cu-O: 1.93



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C.Chlorella     MPCPVCKDGCKCPPGGCACGAECTCCK-AAPCEKCKDKCECGANCD----CKGNCGCCGGK 56
S.elongatus     --TSTTLVKCACEP--CLCNVDPSKAIDRNGLYYCSEACADGHTGGSKGCGHTGCNCHG-- 55
S.platensis     -MTTVTQMKCACES--CLCIVDTTKAIAKNGQYYCSEACANGHPNG-DGCGHKGCNCHH-- 55
P.tenue         -MTTVTQMKCACDS--CLCVVDTSQAVEKDGHYFCSEACANGHPEGSAGCGHPGCGCNS-- 56
                    .    * *    * * .: : .        *.: *  *   .     : .*.*
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Fig.5. Multiple sequence alignment of MT sequences with *Synechococcus elongatus* Mt sequence. The conserved residues are represented with *. The cysteine-rich motifs are observed in the figure.

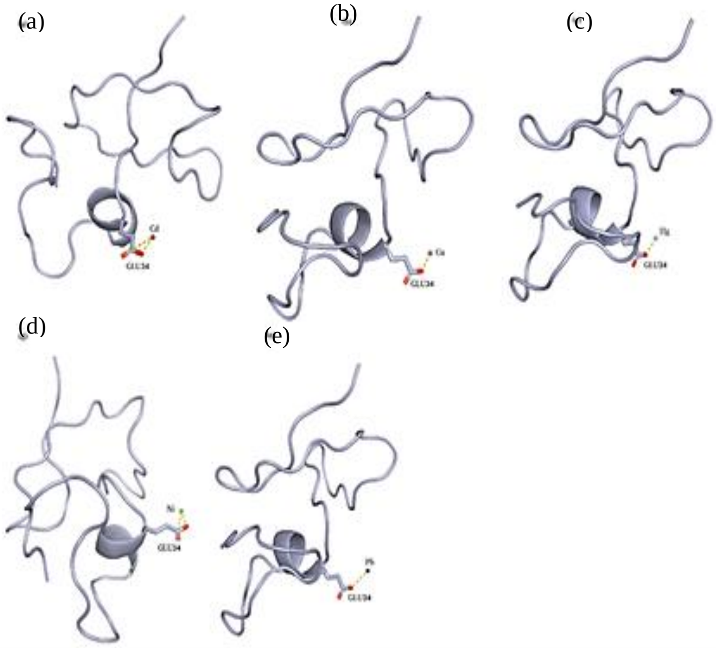

Fig. 6. Protein-metal complexation in *S. Platensis*. Mt protein is shown in cartoon, and the metal ion is represented as a sphere: (a) cadmium (cd), (b) copper (Cu), (c) mercury (Hg), (d) nickel (Ni), and (e) lead (Pb).

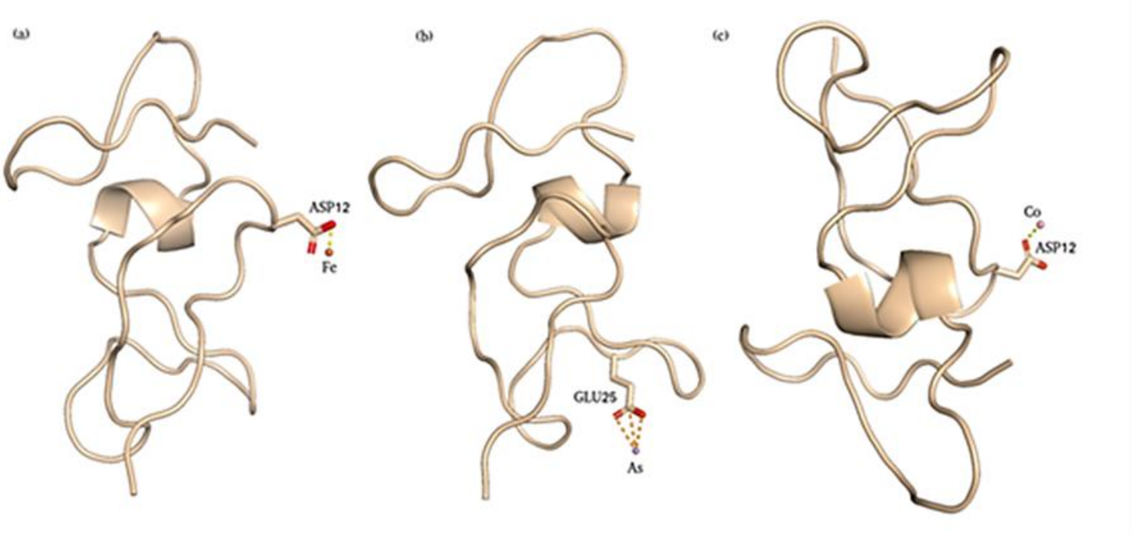

Fig 7. Protein-metal complexation in *P. tenue*. Mt protein is shown in cartoon, and the metal ion is represented as sphere: (a) iron (Fe), (b) arsenic (As), and (c) cobalt (Co). Yellow dashes indicate metal coordination and orange dashes indicate steric clashes.

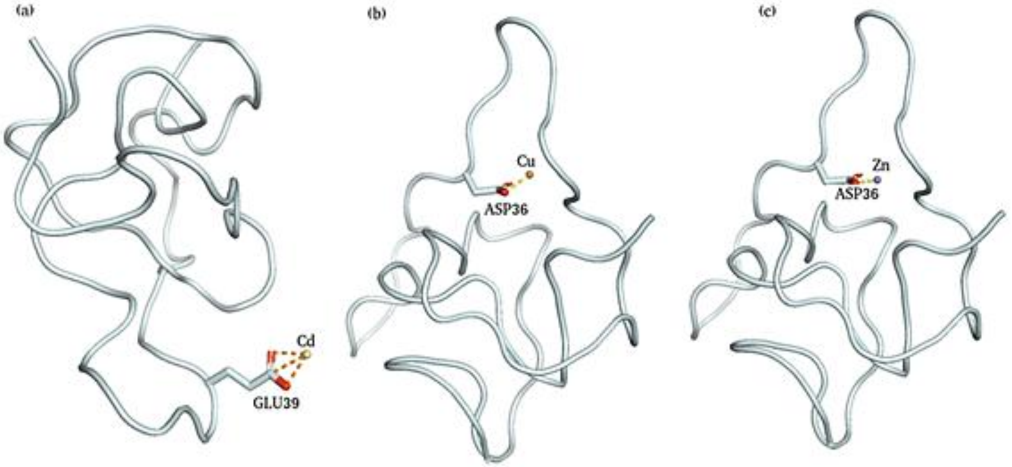

Fig. 8. Protein-metal complexation in *C. Sorokiniana*. Mt protein is shown in cartoon, and the metal ion is represented as a sphere: (a) cadmium (Cd), (b) copper (Cu), and (c) zinc (Zn). Yellow dashes indicate metal coordination and orange dashes indicate steric clashes

8.2.5 Implications for phycoremediation

Taken together, the docking results indicate that microalgal MTs provide a versatile coordination environment based on combinations of cysteine thiolates, carboxylate side chains (Asp/Glu), and, in some cases, histidine imidazoles, providing a versatile and selective coordination environment capable of stabilizing a broad range of divalent and trivalent metal ions. The markedly stronger affinities predicted for *P. tenue* MT, followed by *C. sorokiniana* and *S. platensis,* suggested species-specific potential for heavy metal sequestration that could be exploited when selecting or engineering strains for phycoremediation of metal-contaminated wastewaters.

9. Future Research Directions

This review work concentrated on the technological opportunities and limitations of microalgae-based wastewater treatment. Traditional (physicochemical and biological) methods are widely employed to treat wastewater but are not practical from an economic standpoint. Additionally, microalgae are cost-effective, environmentally friendly, and renewable sources of biofuels and bioactive goods. It is also essential to conduct ongoing investigations to better understand the underlying mechanisms and to develop more thorough equilibrium and kinetic models for the biosorption and bioaccumulation of heavy metals by microalgae. Due to their potential for industrial use, methods for removing and recovering heavy metals, including microalgal biofilm, whole-cell immobilization, and pelletization, have attracted significant attention [211]. Specifically, microalgal fatty acid composition can be altered by heavy metal stress to create biodiesel with desired qualities and characteristics. However, the downstream processing of these valuable products may be hampered by the presence of heavy metals. Therefore, in order to achieve adequate simultaneous heavy metal bioremediation and value-added product production, more studies are needed on upstream cultivation and downstream purification processes [212]. Future research should focus on developing highly efficient methods that lower heavy metal pollution and support long-term, sustainable economic growth. Hazardous heavy metal ions should be eliminated from water systems using a waste-to-resource strategy. The concepts and mechanisms of heavy metal complex breakdown and heavy metal ion recovery via advanced oxidation processes require further study [213]. Selecting environmentally acceptable technology that can be scaled up for real-world applications at a reasonable cost is crucial for the effective and efficient removal of heavy metals.

Future research in microalgal-based bioremediation should focus on enhancing metal tolerance and uptake through genetic engineering, optimizing growth conditions, and understanding multi-metal interactions in real-world environments. Advances in synthetic biology could enable the overexpression of metallothioneins, phytochelatins, and metal transport proteins, improving the sequestration capacity of microalgae. Additionally, most studies focus on single-metal removal, while contaminated environments often contain complex mixtures of pollutants. Investigating synergistic and antagonistic effects among multiple heavy metals and emerging contaminants, such as pharmaceuticals and nanomaterials, is crucial. Optimizing cultivation conditions and designing cost-effective bioreactors will also enhance large-scale applications by improving biomass productivity and metal removal efficiency.

Furthermore, omics-based approaches, including transcriptomics, proteomics, and metabolomics, can provide deeper insights into the molecular and biochemical pathways underlying metal detoxification in microalgae. Integrating bioremediation with circular economy principles by repurposing metal-laden biomass for biofuel production, biofertilizers, or nanoparticle synthesis could add economic value and promote sustainability. Field-scale validation remains a major gap, as most research is conducted under controlled laboratory conditions. Large-scale trials in real-world settings are necessary to assess long-term stability and practical feasibility. Collaboration between academia, industry, and policymakers will be essential for the commercialization and regulatory approval of microalgal bioremediation technologies, ultimately making them viable solutions for mitigating heavy metal pollution in aquatic ecosystems.

10. Conclusion

Algae are widely studied for their potential, means of metal accretion, simulating metal surface assimilation, and inherent modification, as well as their ability to accumulate, adsorb, and recover HMs. Metal binding by microalgae is mediated by a variety of functional groups, including macromolecules and polypeptides. Phytoremediation and biosorption of heavy metals involve mechanisms such as extracellular surface assimilation, reduction, ion exchange, intracellular accumulation, chelation, and bio-methylation. Several biological and abiotic aspects influence the performance of microalgal remediation technologies, including tolerance, strain, size, life stages, metal type and size, atomic weight, concentration, and other environmental factors. Noteworthy technological developments are now proposing the improvement of biomass management using immobilization techniques, algal biosensors in heavy metal identification, along with the strategic use of transgenic microalgae. Microalgae are acknowledged to execute well at a small intensity of defilement, as they don't generate noxious slush; they are effortless cultured and sustained, possess a high interacting affinity (due to their relatively high specific surface area and net negative charge), and are well-suited for both small-scale and large-scale remediation strategies.

This review article explores the potential of microalgae as a sustainable and cost-effective solution for heavy metal bioremediation, offering an alternative to conventional treatment methods. It discusses the natural and anthropogenic sources of heavy metal contamination and their toxic effects on ecosystems and human health. The article highlights various microalgal mechanisms for heavy metal removal, including biosorption, bioaccumulation, enzymatic detoxification, and metal sequestration. Additionally, it incorporates computational analyses of metallothionein (MT) proteins, revealing insights into their metal-binding affinities. A comparative evaluation of different microalgal species and their metal-removal capabilities is presented, emphasizing their effectiveness in remediating pollutants, such as cadmium, lead, chromium, zinc, copper, arsenic, and mercury. The review also identifies key future research directions, such as genetic engineering to enhance metal uptake, large-scale bioreactor optimization, omics-based studies, and integration with circular-economy strategies for resource recovery. In conclusion, the article underscores the significance of microalgae in wastewater treatment and environmental restoration while calling for field-scale validation, industrial collaboration, and regulatory frameworks to facilitate widespread adoption of microalgal-based remediation technologies.

Abbreviations

Chromium (Cr), Copper (Cu), Zinc (Zn), Lead (Pb), Mercury (Hg), Arsenic (As), Iron (Fe), Cadmium (Cd), Heavy Metals (HMs), Central Nervous System (CNS), Total Dissolved Solids (TDS), Total Suspended Solids (TSS Reactive Oxygen Species (ROS) , Fourier-Transform Infra-Red Spectroscopy (FTIR), X-ray Photoelectron Spectroscopy (XPS),Metallothionine (MT)

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