IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Recent Advancement Of Heavy Metals Bioremediation Using Algae: Mechanism And Challenges

Khushaboo Soni & Sanjay Singh*

Department of Botany, CMP Degree College,
University Of Allahabad
Prayagraj 211002 Uttar Pradesh, India

Abstract

The environment and all land, air, and water-based life are seriously threatened by drinking water and wastewater that include heavy metals. Various traditional, cutting-edge nanomaterials-based and biological methods have been used to treat heavy metals. Microalgae are a significant class of microorganisms that can remove heavy metals from wastewater and are useful for a wide range of environmental applications. Additionally, it differs from traditional remedial techniques in many positive ways. As a nutrition source to control its metabolic process for biomass generation, microalgae cells are used to ingest the heavy metal in various physiological and biological ways. A variety of techniques, including the immobilization of algal cells, the formation of algal consortiums, and the production of nanomaterials based on microalgae can also be used to increase the effectiveness of heavy metal removal. Additionally, it can make a substantial contribution to the future and sustainability of the environment. Thus, the paper offers a comprehensive overview of heavy metals, their existence, and their detrimental consequences on people. This paper also offers information on summaries of microalgal-based heavy metal absorption mechanisms and their potential for amputation of various heavy metals. Additionally, a particular emphasis is placed on contemporary methods that improve the effectiveness of heavy metal removal and contribute to sustainability for the advancement of a microalgae-based future.

Keywords: Heavy metals, Bioremediation, Microalgae, Water pollution

Introduction

The risk of water contamination has increased as a result of urbanization, industrialization, and intensive human activity. Heavy metals, which are inherently non-thermally and non-biodegradable, have been released into the environment as a result of the fast expansion of industry and human activity. Rivers, lakes, and oceans have been harmed by residual heavy metal ions from household and business runoff. In the Hubei area of China, well water was found to have significant amounts of copper and cadmium [Cai et al. 2015]. By lowering plant growth and yield metrics, heavy metal stress puts crops in jeopardy [Liu et al. 2016]. Heavy metals make organic pollutants less biodegradable, extending their environmental persistence and aggravating the effects of other hazardous wastes [Briffa et al. 2020]. These metal ions are very mobile in aquatic systems and are thought to be exceedingly harmful even in tiny concentrations because they are non-essential [Nateras-Ramrez et al. 2022]. Through biosorption and buildup in the aquatic food chain, humans may be receiving heavy metal ions, which can cause serious health issues, organ tissue damage, and cell degradation from overconsumption [Jaishankar et al. 2014].

The crust and soil of the planet contain naturally occurring heavy metals. Elements with metallic characteristics and an atomic density of more than 4 g cm^{-3} are referred to as heavy metals. Additionally, it is five times denser than water and about 53 distinct compounds are classified as heavy metals. According to Shanab et al. (2012) and Kumar et al. (2015), it has an atomic weight range of 63.5 to 200.6 and a specific gravity greater than 5.0. According to Kumar et al. (2015), heavy metals are defined as any metallic chemical compound with a high density, that is hazardous, does not degrade biologically, and pollutes the environment. However, a small number of these heavy metals constitute significant sources of nutrients for plants, microorganisms, and mammals at lower concentrations [Kumar et al. 2015].

To remove heavy metals from contaminated water, various techniques are being used, including chemical precipitation, adsorption, electrodialysis, ion exchange, membrane filtration, coagulation/flocculation, and electrochemical precipitation [Chen et al. 2023]. Each of these approaches has benefits and drawbacks of its own. The development of novel, environmentally benign, and long-lasting techniques that can guarantee the total removal of heavy metals is of great significance in light of the encouragement of environmental sustainability [Chen et al. 2023]. Through a sequence of physicochemical interactions between the functional groups of microorganisms and the heavy metals, bioremediation is an environmentally benign method that leverages the metabolic potential of microorganisms to remove heavy metals [Wang et al. 2021]. One of the best techniques for eliminating radioactive ions and heavy metals from wastewater is biosorption. Biosorption offers the potential for waste material recycling in addition to being economically advantageous [Jiang et al. 2013]. Algae are a naturally occurring biomass, and they vary in their affinity for heavy metals [Ahmad et al. 2019]. [Chen et al. 2014] Algal biosorption improves the effectiveness, efficiency, and environmental safety of heavy metal ion removal from wastewater. Algae are used as an alternative to traditional treatment methods for a variety of industrial, agricultural, and mining wastewaters because of their potential for nutrient removal, adsorption, and regenerative and sustainable nature [Xia et al. 2016]. Because algae can absorb CO2 through photosynthetic processes and can adapt to growing in many types of wastewater, algae growth in aquatic systems is an eco-friendly, greener, and sustainable bioremediation approach [Bhatt et al. 2022]. Algal cells can adhere to particular surfaces, and this ability to generate biomembranes lowers the overall cost of separating algal biomass from the rest of the medium [Mandotra et al. 2020]. The normal harvesting and dewatering procedure can be simplified and made more energy-effective by using larger immobilized microalgae beads or carriers. Immobilized algal cultures may lessen the disruption of the native ecosystem brought on by the introduction of foreign microorganisms since microbeads may stop the discharge of immobilized microorganisms into wastewater [Covarrubias et al. 2012].

Microalgae are a strong contender for the bioremediation of polluted water [Goswami et al. 2021b, 2022] and heavy metals due to their increased removal efficiency and biosorption and bioaccumulation mechanisms [Leong et al. 2020]. Additionally, the biomass that is produced contains a variety of compounds with added value that can be used to create bioenergy products. The importance of microalgae in the bioremediation of heavy metals, their heavy metals uptake mechanism, and future aspects of bioremediation are all thoroughly analyzed in this paper. Additionally, this review offers useful information on current methods for removing heavy metals from microalgal sources.

Existence of Heavy Metals in Wastewater

Monteiro et al. (2012) proposed a list of major industries, including (i) metalliferous mining industries that discharge acid mine residues and drainage containing Cd, Cu, Cr, Ni, Co, and Zn; (ii) manure sewage sludge containing Zn, Ni, Pb, Cd, etc.; (iii) fertilizers industries that discharge Cd, Cr, Mo, Pb, U, V, and Zn in surface and groundwater; and (iv) specialist alloys and steels industry that manufacture, dispose of, recycle metals and release Pb, Mo, Ni, Cu, Cd, As, Te and Zn; (v) Aqueous waste released from paints and pigments that manufacture, old paint deterioration and soil pollution containing Pb, Cr, As, Ti, Ba, Zn, and Cd; (vi) liquid effluents released from electroplating industries and plating processes containing Cr, Ni, Zn, Cu, and Cd and (vii) waste disposal release landfill leachate, contaminate ground and surface waters containing Zn, Cu, Cd, Pb, Ni, Cr and Hg. Therefore, heavy metal enrichment in water is caused by a variety of industries, including those in the chemical, mining, smelting, metallurgical, plastic, paint, textile, and clothing industries, as well as the printing, battery, ceramic, steel production, agrochemical, animal feed, paper-pulp, and fiber industries [Kumar et al. 2015].

Heavy Metals Destructive Properties

Because of the food chain, heavy metals that are transported from the water system to plants and people might endanger the health of the ecosystem's living things. Heavy metal contamination in drinking water can be extremely poisonous and unhealthy for living things. The maximum contamination levels for heavy metals in drinking water, as stated by the U.S. EPA (2009), are Hg (0.002 mg l^{-1}), Pb (0.015 mg l^{-1}), Cr (0.1 mg l^{-1}), Cu (1.3 mg l^{-1}), Cd (0.005 mg l^{-1}), and Zn and Ni (0.04 mg l^{-1}). According to Kumar et al. (2015), exposure to certain heavy metals at levels over the allowable limits can seriously harm aquatic life, humans, and the soil's fertility. Ingesting Cr can result in nausea, skin irritation, kidney damage, lung disease, and other conditions [Cheng et al. 2019]. On the other hand, ingesting Zn can result in dizziness, nausea, skin irritation, and other conditions. In addition, exposure to heavy metals including Ce^{3+}, Co^{2+}, Cs^{2+}, Cu^{2+}, Hg^{2+}, Ln^{3+}, and Pd^{2+} can result in several syndromes and diseases like Parkinson's disease and Alzheimer's disease as well as liver damage and neurological problems [Alam et al. 2019].

Heavy Metal Elimination Using Various Traditional Methods

Researchers have used various techniques for removing heavy metals from various wastewater for a long time. These techniques can be divided into electrochemical treatments (electrocoagulation, elector-floatation, and electrodeposition), physicochemical processes (chemical precipitation, Ion exchange), adsorption (activated carbon, carbon nanotubes, and wood sawdust adsorbents), or contemporary techniques (membrane filtration processes, photocatalysis processes, and nanotechnology [Arezoo et al. 2017].

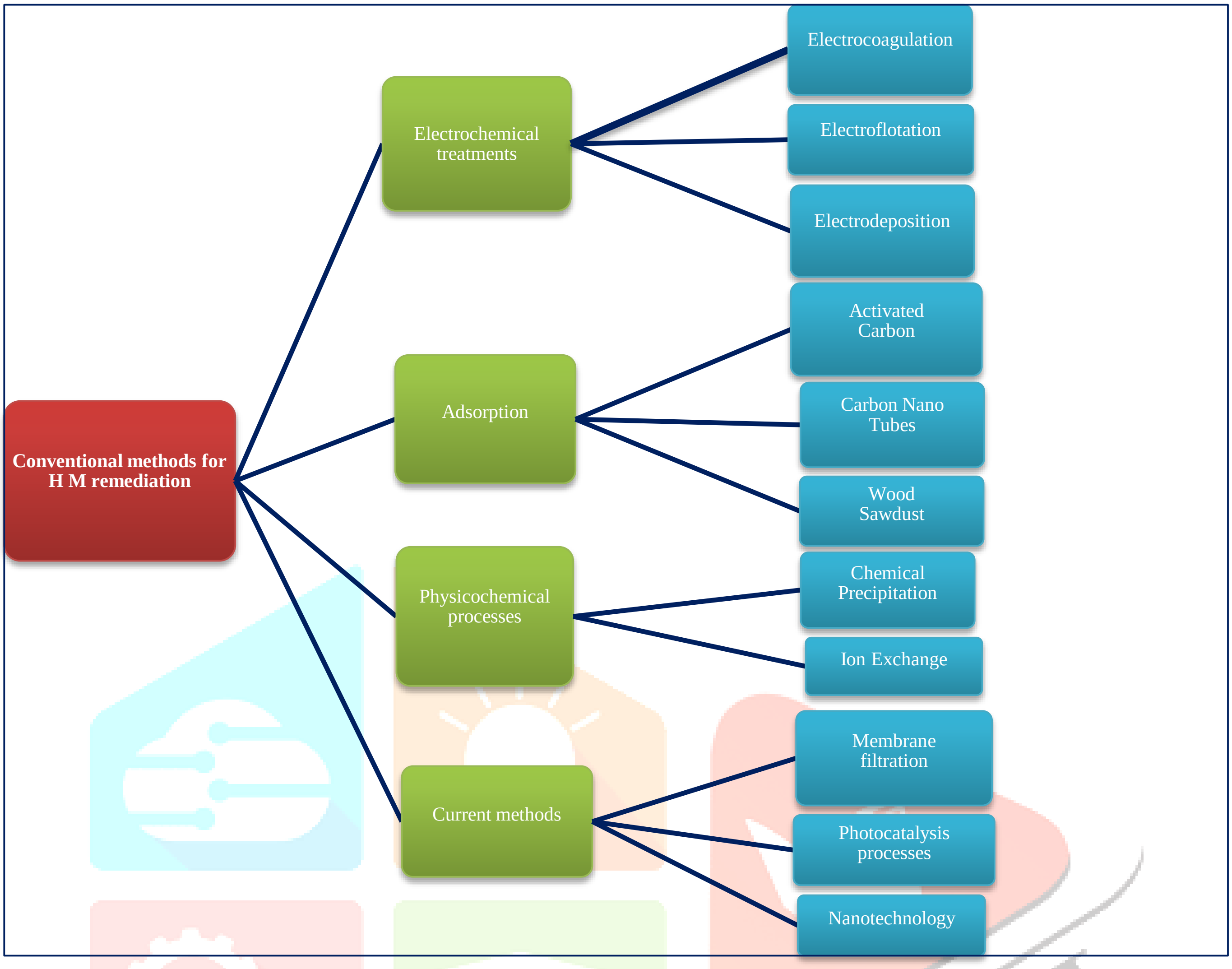

Fig 1: Conventional methods for heavy metal remediation

The potential of Different Microalgae for Removing Heavy Metals

Aquatic environments are home to eukaryotic, phototrophic microorganisms known as microalgae. The process of photosynthesis, fixes carbon dioxide from the environment and controls its metabolism [Chaturvedi et al. 2021; Goswami et al. 2021]. The microalgae could purify wastewater and lessen its nutrient content. Diverse microalgae have been applied to wastewater treatment globally over the past three decades [Samal et al. 2020]. As shown in Table 1, numerous published studies hypothesized that microalgae can remove heavy metals [Samal et al. 2020]. In their experiment, Pena-Castro et al. (2004) used the microalga *Scenedesmus incrassatulus* to remove chromium (VI), cadmium (II), and copper (II). The results showed that *S. incrassatulus* removed 25-78% of the heavy metals, with chromium (VI) being the metal that was most significantly eliminated. Similar to Omar (2002), who investigated the removal of Zn using *Scenedesmus quadricauda* and *S. obliquus*, the experimental results showed that *S. obliquus* had greater removal effectiveness than *S. quadricauda*. Moreover, Soeprobowati and Hariyati (2012) studied the potential of microalgae *Chlorella sp.*, *Spirulina sp.*, *Chaetoceros sp.*, and *Porphyridium sp*. for the removal of heavy metals such as Cd, Cu, and Pb (at the concentration of <05 mg l1) in which *Chlorella sp.* showed highest removal efficiency of Pb (90%), Cd (62%) and Cu (83%), *Porphyridium sp.* showed the highest reduction of Cd (70%) and Cu (96%). Additionally, Goswami et al. (2022) investigated the capacity of various *Picochlorum* strains to remove heavy metals from various types of wastewaters. These findings suggested that the efficacy of heavy metals reduction varies depending on the species of microalgae and is also influenced by the types of heavy metals present in the culture medium or wastewater. Adsorption and desorption techniques can be used to remove heavy metals from dead microalgae cells and compounds derived from microalgae. In addition, Cheng (2017) used both live and dead *C. vulgaris* to study the biosorption and kinetics of Cd (II) removal. According to the findings, *C. vulgaris* has a high effectiveness of Cd adsorption in both dead cells (96.8%) and live cells (95.2%). Additionally, Shokri Khoubestani et al. (2015) performed batch mode studies to remove Cr (III) and Cr (VI) and assessed the impact of pH on a biosorbent made from microalgae biomass that contained protein (43.5%), carbohydrate (20.2%), and lipid (92%). The results showed that Cr (III) absorption was 98.3% at pH 6, while Cr (VI) absorption was 47.6% at pH 1. To study the mechanism of Pb (II) elimination, Danouche et al. (2021) used the Pb (II)-tolerant microalga *S. obliquus*. The findings demonstrated that *S. obliquus* ingested Pb (II) intracellularly or extracellularly through biosorption (85.5%) and bioaccumulation (14.5%).

Table 1: Different microalgae were reported to remove different types of heavy metals

Microalgae	Heavy metal and its removal efficiency	References
Chlorella sp.	Pb: 90%, Cd: 62%, Cu: 83%	Soeprobowati and Hariyati (2012)
Chaetoceros sp.	Pb: 81%	Soeprobowati and Hariyati (2012)
Porphyeridium sp.	Cd: 70%, Cu: 96%	Soeprobowati and Hariyati (2012)
Spirulina sp.	Cd: 73%	Soeprobowati and Hariyati (2012)
Scenedesmus sp.	Cr(VI): 92.89%	Pradhan et al. (2019)
Pseudochlorococcum typicum	Hg^{2+}: 97%, Pb^{2+}: 70%, Cd^{2+}: 86%	Shanab et al. (2012)
Chlorella minutissima UTEX 2341	Cu: 83.68%, Mn: 83.68%, Cd: 74.34%, Zn: 62.05%	Yang et al. (2015)
Desmodesmus sp. MAS1 and *Heterochlorella sp.* MAS3	Cd: >58%	Abinandan et al. (2019)
Chlorella vulgaris	Cu: 39%, Ni: 32%	Rugnini et al. (2017)
Desmodesmus sp.	Cu: 43%, Ni: 39%	Rugnini et al. (2017)
Chlamydomonas reinhardtii	As: 38.6%	Saavedra et al. (2018)
Scenedesmus almeriensis	As: 41.7%	Saavedra et al. (2018)

Uptake Mechanisms of Different Heavy Metals via Microalgae

Algae are capable of creating cellular protein-heavy metal complexes without altering their activity [Priatni et al. 2018]. To regulate the concentration of heavy metal ions in the cytoplasm and decrease their harmful effects, organometallic complexes are further split inside the vesicles [Balaji et al. 2016]. Algae can remove heavy metals from the environment using a three-stage mechanism that involves extracellular precipitation or accumulation of heavy metals by living cells, complexation or cellular adsorption in living and dead cells, and intracellular internalization requiring microbial activity or metabolic processes [Leong et al. 2020].

Living and non-living biomass can both perform biosorption processes, also referred to as fast extracellular passive processes. Biosorption, which is an effective way to remove heavy metals from industrial effluent, is the main mechanism of heavy metal adsorption by active or passive algal biomass [Ahmad et al. 2020]. Heavy metal ions interact with negatively charged functional groups on the algal cell surface for a short period before being passively absorbed. Sulfate, carboxyl, amino, and hydroxyl groups found in cell walls are binding sites for heavy metals. Heavy metal ions are then attached to these functional groups via chelation/complexation, adsorption, electrostatic interactions, surface precipitation, and ion exchange [Park et al. 2016]. It has become the primary approach that positively or negatively charged ions will bind to the negatively charged biosorbent's surface [Chojnacka et al. 2010]. The protonation of the functional groups on algal biomass particles and the amino and hydroxyl groups on carriers may affect the electrostatic repulsion between positively charged surfaces and metal cations [Sargin et al. 2016]. Furthermore, biosorption can assemble complexes with functional groups that are present on the surface of cells [Saba et al. 2018]. Algae's cyanobacterial extracellular polymer components include a wide range of biopolymers, including humic substances, lipids, nucleic acids, polysaccharides, proteins, and glyoxylates [Aswathi Mohan et al. 2022]. A key role in the biosorption of heavy metals is played by cyanobacterial extracellular polymers, which also operate as a protective barrier against harmful external elements [Greeshma et al. 2022]. Heavy metals can easily bind to the lipids, proteins, and surfaces of algae thanks to polysaccharides. Moreover, when the pH of the solution rapidly shifts due to biosorption or when the metal concentration reaches saturation, heavy metals have the propensity to precipitate and build up on the cell surface. Algae can attach to heavy metals in this manner in addition to other methods.

It takes energy to move heavy metals across the cell membrane to the cytoplasm or other organelles, and this process, known as active bioaccumulation, leads to the accumulation of intracellular heavy metals [Zohoorian et al. 2020]. Chemicals and nutrients are absorbed through the surface of the biomass, which either accumulates or metabolizes substances, depending on the type of biomass. The entire process, which takes a long time from the absorption of metal ions to the transit of these ions throughout the cell or any organelle [Chugh et al. 2022], depends on ion-selective transport proteins that are located in the cell membrane. Algae must protect cells from non-essential metals and keep intracellular ion concentrations within normal ranges. The host cell is spared the inhibitory effects of a large concentration of metal ions because structural/binding proteins, like metallothioneins, attach to the adsorbed ions [Tripathi et al. 2021]. Metal binding occurs as organometallic complexes that are preserved in the organelles of microalgal cells thanks to the sulfhydryl groups in phytochelatin peptides produced by microalgae through enzymatic synthesis [Ahmad et al. 2019]. Acidic calcifiers and polyps also encourage the buildup and storage of heavy metals [García-García et al. 2016].

Although detoxification routes in algae have also been used, biotransformation in algae is mostly applied to the enzymatic and biochemical transformation of heavy metals. Heavy metals' inability to degrade leads to enzymatic biotransformation, which transforms them into less hazardous inorganic complex forms [Pradhan et al. 2022]. Biotransformation, on the other hand, uses electron transfer to reduce extremely valuable heavy metals, which are then transformed into organic heavy metal compounds [Yen et al. 2017]. Additionally, because heavy metal ions have a variety of characteristics, distinct algal adsorption methods may exist [Sarojini et al. 2021]. According to Raize (2004), chelation appears to be the main mechanism for the adsorption of cadmium cations by algal biomass and ion exchange for the adsorption of nickel ions. In contrast, the precipitation of metallic lead on algal biomass is combined with ion exchange, chelation, and reduction processes during the binding processes of lead cations. According to Abdel-Aty (2013), lead cations have a stronger affinity for algal biomass. Sarojini et al. [Sarojini et al. 2021] confirmed that ion exchange and electrostatic interactions are the primary mechanisms by which algae absorb Cr ions. Microalgae oxidize As (III) to As(V), which is then converted into less hazardous forms through methylation, volatilization, and extracellular excretion [Zhang et al. 2014]. Increased Cd levels significantly affect cellular functions related to energy use, DNA replication, cell cycle, and signal transduction [Badisa et al. 2007].

Recent Advanced Strategies in Microalgal-based Heavy Metal Remediation

Heavy metals can be removed by microalgae using either living or dead cells. To improve the efficiency of removal and biomass use, more system modification is necessary, according to Cheng et al. (2019). The immobilization of microalgal biomass for the remediation of heavy metal-polluted waterbodies has been the subject of numerous types of research. For instance, *Tetraselmis chuii* was immobilized using Ca-alginate by Moreno-Garrido et al. (2005) to remove Cd and Cu from seawater. The results showed that the immobilized cells removed 20 and 100% Cd and Cu, respectively. It may be possible to remove heavy metals using a loofa sponge or an immobilized alga cell made of *Lufa cylindrica*. *Chlorella sorokiniana* cells immobilized on *L. cylindrica* and free *C. sorokiniana* cells were both tested for their ability to remove Cd by Akhtar et al. in 2003. According to the results, immobilized *C. sorokiniana* cells removed 97.9% of the Cd at an initial concentration of 10 mg l-1, but free *C. sorokiniana* cells removed 92.7% of the Cd. According to this investigation, immobilizing microalgae improved the recovery effectiveness of heavy metals. Moreover, Shen et al. (2020) proposed that microalgae can immobilize and recycle Cd ions using a bioreactor system. According to reports, a mixture of metal oxides, like Fe_2O_3, also achieves the goal of immobilizing ions and aids in the removal of heavy metal with a higher efficiency rate. Due to their numerous applications, the production of nanoparticles from microalgal biomass is receiving a lot of attention. It is smaller, more reactive, has a greater surface-to-volume ratio, etc. In recent times, it has been possible to use the adsorption technique to remove heavy metals (Cheng et al. 2019). These methods are also employed to get rid of heavy metals. *Spirulina platensis*, *C. vulgaris*, and *Scenedesmus quadricuda* were used by Abdel-Razak et al. (2019) to remove heavy metals like Ni, Cd, and Pb as well as the organophosphate pesticide malathion from water is a mixture of urban and agricultural wastewater. The results showed that a consortium of microalgae cleared a sample of water of Ni (95%), Pb (89%), Cd (88%), and malathion (99%). When Yang et al. (2021) assessed the removal of Cr (VI) using a related technique, they hypothesized that exopolysaccharides released by algal bacteria were loosely bound and favored Cr (VI) remediation. The adsorption of heavy metals has depended greatly on pH, as was indicated in the section before this one. Similar to this, the pH of alga-bacterial consortia plays a big part in the removal of heavy metals. Yang et al. (2021) also noted that the best pH for the reduction of Cr (VI) in algal-bacterial granular sludge was 2, whereas pH 6 was necessary for the removal of all forms of Cr.

Challenges and Proposed Strategies of Microalgae-based Heavy Metal Bioremediation

A replacement biosorbent for heavy metal remediation can be made from microalgae biomass due to its high capacity for metal biosorption. It has several disadvantages, including tiny particle size, poor chemical resistance, weak mechanical strength, and difficulty in separating algal biomass from contaminated water [Salam et al. 2019]. The following is the suggested strategy for producing biofuels and bioremediating heavy metals: (i) Selection of an appropriate microalgal strain with high metal tolerance and rapid growth, (ii) ability to synthesize high levels of lipid and other metabolites when heavy metals are present, (iii) ability to grow with low nutrient requirements and have high CO2 sequestration ability and (iv) resistance and dominance over other microorganisms that are present in the medium or wastewater are all necessary for remediation purposes [Leong et al. 2020]. Increasing the effectiveness of heavy metal removal through the immobilization of microalgae may be a sustainable strategy, but the cost of remediation may go up. However, using waste as a means of immobilization could be an option for this issue. The organic and inorganic ligands, chelating agents, and sensors based on nanomaterials are widely used in the removal of the many kinds of heavy metals that have already been described. Microalgae consortiums may also be an appropriate strategy for the removal of heavy metals. Genetic or metabolic engineering is a key tool for changing genes to perform a certain function [Mehariya et al. 2021]. This method aids in enhancing the microalgae strain's capacity for metal tolerance, adaptability, specificity, and robustness [Leong et al. 2020]. A crucial strategy to improve the effectiveness of heavy metal removal may be the insertion of functional group synthesis genes and heavy metal binding protein genes into the microalgae genome [Cheng et al. 2019]. Further study is necessary to comprehend the heavy metal remediation mechanism in microalgae.

Conclusion

Heavy metals in contaminated water are a serious threat to all living things. Bioalgal remediation is considered a practical and environmentally conscious alternative to traditional wastewater treatment procedures since it is less complicated, expensive, and has fewer limits. The most common organisms for heavy metal remediation are microalgae. Microalgae have several significant processes that they can use to remove heavy metals from a water sample. As a result, combining the growing of microalgae with the removal of heavy metals from wastewater can be advantageous for both parties because the resulting biomass contains lipids and other metabolites that can be utilized to make biofuel. Microalgae use the processes of biosorption, bioaccumulation, and biotransformation to remove heavy metals from the environment. Ion exchange, chelation/complexation, electrostatic interactions, and surface precipitation are the mechanisms influencing the adsorption of heavy metals by algae. More research and approaches are needed to increase the removal efficiency and consumption of its biomass to further contribute to the establishment of a future based on microalgae.

References

1. Abdel-Aty, A. M., Ammar, N. S., Ghafar, H. H. A., & Ali, R. K. (2013). Biosorption of cadmium and lead from aqueous solution by fresh water alga Anabaena sphaerica biomass. *Journal of advanced research*, *4*(4), 367-374.
2. Abdel-Razek, M. A., Abozeid, A. M., Eltholth, M. M., Abouelenien, F. A., El-Midany, S. A., Moustafa, N. Y., & Mohamed, R. A. (2019). Bioremediation of a pesticide and selected heavy metals in wastewater from various sources using a consortium of microalgae and cyanobacteria. *Slov Vet*, *56*(Suppl 22), 61-73.
3. Abinandan, S., Subashchandrabose, S. R., Venkateswarlu, K., Perera, I. A., & Megharaj, M. (2019). Acid-tolerant microalgae can withstand higher concentrations of invasive cadmium and produce sustainable biomass and biodiesel at pH 3.5. *Bioresource Technology*, *281*, 469-473.
4. Ahmad, A., Bhat, A. H., & Buang, A. (2019). Enhanced biosorption of transition metals by living Chlorella vulgaris immobilized in Ca-alginate beads. *Environmental technology*, *40*(14), 1793-1809.
5. Ahmad, J., Ali, A. A., Baig, M. A., Iqbal, M., Haq, I., & Qureshi, M. I. (2019). Role of phytochelatins in cadmium stress tolerance in plants. *Cadmium toxicity and tolerance in plants*, 185-212.
6. Ahmad, S., Pandey, A., Pathak, V. V., Tyagi, V. V., & Kothari, R. (2020). Phycoremediation: algae as eco-friendly tools for the removal of heavy metals from wastewater. *Bioremediation of Industrial Waste for Environmental Safety: Volume II: Biological Agents and Methods for Industrial Waste Management*, 53-76.
7. Akhtar, N., Saeed, A., & Iqbal, M. (2003). Chlorella sorokiniana immobilized on the biomatrix of vegetable sponge of Luffa cylindrica: a new system to remove cadmium from contaminated aqueous medium. *Bioresource technology*, *88*(2), 163-165.
8. Alam, M. M., Asiri, A. M., Uddin, M. T., Islam, M. A., Awual, M. R., & Rahman, M. M. (2019). Detection of uric acid based on doped ZnO/Ag 2 O/Co 3 O 4 nanoparticle loaded glassy carbon electrode. *New Journal of Chemistry*, *43*(22), 8651-8659.
9. Azimi, A., Azari, A., Rezakazemi, M., & Ansarpour, M. (2017). Removal of heavy metals from industrial wastewaters: a review. *ChemBioEng Reviews*, *4*(1), 37-59.
10. Badisa, V. L., Latinwo, L. M., Odewumi, C. O., Ikediobi, C. O., Badisa, R. B., Ayuk-Takem, L. T., ... & West, J. (2007). Mechanism of DNA damage by cadmium and interplay of antioxidant enzymes and agents. *Environmental Toxicology: An International Journal*, *22*(2), 144-151.

11. Balaji, S., Kalaivani, T., Sushma, B., Pillai, C. V., Shalini, M., & Rajasekaran, C. (2016). Characterization of sorption sites and differential stress response of microalgae isolates against tannery effluents from Ranipet industrial area—an application towards phycoremediation. *International journal of phytoremediation*, *18*(8), 747-753.
12. Bhatt, P., Bhandari, G., Bhatt, K., & Simsek, H. (2022). Microalgae-based removal of pollutants from wastewaters: Occurrence, toxicity, and circular economy. *Chemosphere*, *306*, 135576.
13. Briffa, J., Sinagra, E., & Blundell, R. (2020). Heavy metal pollution in the environment and their toxicological effects on humans. *Heliyon*, *6*(9), e04691.
14. Cai, L. M., Xu, Z. C., Qi, J. Y., Feng, Z. Z., & Xiang, T. S. (2015). Assessment of exposure to heavy metals and health risks among residents near Tonglushan mine in Hubei, China. *Chemosphere*, *127*, 127-135.
15. Chaturvedi, V., Goswami, R. K., & Verma, P. (2020). Genetic engineering for enhancement of biofuel production in microalgae. *Biorefineries: a step towards renewable and clean energy*, 539-559.
16. Chen, B. Y., Chen, C. Y., Guo, W. Q., Chang, H. W., Chen, W. M., Lee, D. J., ... & Chang, J. S. (2014). Fixed-bed biosorption of cadmium using immobilized Scenedesmus obliquus CNW-N cells on loofa (Luffa cylindrica) sponge. *Bioresource technology*, *160*, 175-181.
17. Chen, Z., Osman, A. I., Rooney, D. W., Oh, W. D., & Yap, P. S. (2023). Remediation of heavy metals in polluted water by immobilized algae: current applications and future perspectives. *Sustainability*, *15*(6), 5128.
18. Cheng, J., Yin, W., Chang, Z., Lundholm, N., & Jiang, Z. (2017). Biosorption capacity and kinetics of cadmium (II) on live and dead Chlorella vulgaris. *Journal of applied phycology*, *29*, 211-221.
19. Cheng, S. Y., Show, P. L., Lau, B. F., Chang, J. S., & Ling, T. C. (2019). New prospects for modified algae in heavy metal adsorption. *Trends in biotechnology*, *37*(11), 1255-1268.
20. Chojnacka, K. (2010). Biosorption and bioaccumulation–the prospects for practical applications. *Environment International*, *36*(3), 299-307.
21. Chugh, M., Kumar, L., Shah, M. P., & Bharadvaja, N. (2022). Algal Bioremediation of heavy metals: An insight into removal mechanisms, recovery of by-products, challenges, and future opportunities. *Energy Nexus*, 100129.
22. Covarrubias, S. A., de-Bashan, L. E., Moreno, M., & Bashan, Y. (2012). Alginate beads provide a beneficial physical barrier against native microorganisms in wastewater treated with immobilized bacteria and microalgae. *Applied microbiology and biotechnology*, *93*(6), 2669-2680.
23. Danouche, M., El Ghachtouli, N., Aasfar, A., Bennis, I., & El Arroussi, H. (2021). Mechanisms of lead (II) phycoremediation using Scenedesmus obliquus: cell physicochemical properties, biochemical composition, and metabolomic profiling.
24. García-García, J. D., Sánchez-Thomas, R., & Moreno-Sánchez, R. (2016). Bio-recovery of non-essential heavy metals by intra-and extracellular mechanisms in free-living microorganisms. *Biotechnology advances*, *34*(5), 859-873.
25. Goswami, R. K., Agrawal, K., & Verma, P. (2021). Microalgae-based biofuel-integrated biorefinery approach as sustainable feedstock for resolving energy crisis. *Bioenergy research: commercial opportunities & challenges*, 267-293.
26. Goswami, R. K., Agrawal, K., & Verma, P. (2022). Phycoremediation of nitrogen and phosphate from wastewater using Picochlorum sp.: A tenable approach. *Journal of Basic Microbiology*, *62*(3-4), 279-295.
27. Goswami, R. K., Mehariya, S., Obulisamy, P. K., & Verma, P. (2021). Advanced microalgae-based renewable biohydrogen production systems: A review. *Bioresource Technology*, *320*, 124301.
28. Greeshma, K., Kim, H. S., & Ramanan, R. (2022). The emerging potential of natural and synthetic algae-based microbiomes for heavy metal removal and recovery from wastewater. *Environmental Research*, *215*, 114238.
29. Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B. B., & Beeregowda, K. N. (2014). Toxicity, mechanism, and health effects of some heavy metals. *Interdisciplinary toxicology*, *7*(2), 60.
30. Jiang, W., Xu, Y., Li, C., Lv, X., & Wang, D. (2013). Biosorption of cadmium (II) from aqueous solution by chitosan encapsulated Zygosaccharomyces rouxii. *Environmental Progress & Sustainable Energy*, *32*(4), 1101-1110.

31. Kumar, K. S., Dahms, H. U., Won, E. J., Lee, J. S., & Shin, K. H. (2015). Microalgae–a promising tool for heavy metal remediation. *Ecotoxicology and environmental safety*, *113*, 329-352.
32. Leong, Y. K., & Chang, J. S. (2020). Bioremediation of heavy metals using microalgae: Recent advances and mechanisms. *Bioresource technology*, *303*, 122886.
33. Liu, L., Zhang, X., & Zhong, T. (2016). Pollution and health risk assessment of heavy metals in urban soil in China. *Human and ecological risk assessment: An international journal*, *22*(2), 424-434.
34. Mandotra, S. K., Lolu, A. J., Kumar, S., Ramteke, P. W., & Ahluwalia, A. S. (2020). Integrated approach for bioremediation and biofuel production using algae. *Restoration of wetland ecosystem: a trajectory towards a sustainable environment*, 145-160.
35. Mehariya, S., Goswami, R. K., Karthikeysan, O. P., & Verma, P. (2021). Microalgae for high-value products: A way towards green nutraceutical and pharmaceutical compounds. *Chemosphere*, *280*, 130553.
36. Mohan, A. A., Antony, A. R., Greeshma, K., Yun, J. H., Ramanan, R., & Kim, H. S. (2022). Algal biopolymers as sustainable resources for a net-zero carbon bioeconomy. *Bioresource Technology*, *344*, 126397.
37. Monteiro, C. M., Castro, P. M., & Malcata, F. X. (2012). Metal uptake by microalgae: underlying mechanisms and practical applications. *Biotechnology progress*, *28*(2), 299-311.
38. Moreno-Garrido, I., Campana, O., Lubián, L. M., & Blasco, J. (2005). Calcium alginate immobilized marine microalgae: experiments on growth and short-term heavy metal accumulation. *Marine pollution bulletin*, *51*(8-12), 823-829.
39. Nateras-Ramírez, O., Martínez-Macias, M. R., Sánchez-Machado, D. I., López-Cervantes, J., & Aguilar-Ruiz, R. J. (2022). An overview of microalgae for Cd2+ and Pb2+ biosorption from wastewater. *Bioresource Technology Reports*, *17*, 100932.
40. Omar, H. H. (2002). Bioremoval of zinc ions by Scenedesmus obliquus and Scenedesmus quadricauda and its effect on growth and metabolism. *International biodeterioration & biodegradation*, *50*(2), 95-100.
41. Park, D. M., Reed, D. W., Yung, M. C., Eslamimanesh, A., Lencka, M. M., Anderko, A., ... & Jiao, Y. (2016). Bioadsorption of rare earth elements through cell surface display of lanthanide binding tags. *Environmental Science & Technology*, *50*(5), 2735-2742.
42. Pena-Castro, J. M., Martınez-Jerónimo, F., Esparza-Garcıa, F., & Canizares-Villanueva, R. O. (2004). Heavy metals removal by the microalga Scenedesmus incrassatulus in continuous cultures. *Bioresource technology*, *94*(2), 219-222.
43. Pradhan, B., Bhuyan, P. P., Nayak, R., Patra, S., Behera, C., Ki, J. S., ... & Jena, M. (2022). Microalgal Phycoremediation: A Glimpse into a Sustainable Environment. *Toxics*, *10*(9), 525.
44. Pradhan, D., Sukla, L. B., Mishra, B. B., & Devi, N. (2019). Biosorption for removal of hexavalent chromium using microalgae Scenedesmus sp. *Journal of Cleaner Production*, *209*, 617-629.
45. Priatni, S., Ratnaningrum, D., Warya, S., & Audina, E. (2018, June). Phycobiliproteins production and heavy metals reduction ability of Porphyridium sp. In *IOP Conference Series: Earth and Environmental Science* (Vol. 160, No. 1, p. 012006). IOP Publishing.
46. Raize, O., Argaman, Y., & Yannai, S. (2004). Mechanisms of biosorption of different heavy metals by brown marine macroalgae. *Biotechnology and Bioengineering*, *87*(4), 451-458.
47. Rugnini, L., Costa, G., Congestri, R., & Bruno, L. (2017). Testing of two different strains of green microalgae for Cu and Ni removal from aqueous media. *Science of The Total Environment*, *601*, 959-967.
48. Saavedra, R., Muñoz, R., Taboada, M. E., Vega, M., & Bolado, S. (2018). Comparative uptake study of arsenic, boron, copper, manganese, and zinc from water by different green microalgae. *Bioresource Technology*, *263*, 49-57.
49. Salam, K. A. (2019). Towards sustainable development of microalgal biosorption for treating effluents containing heavy metals. *Biofuel Research Journal*, *6*(2), 948.
50. Samal, D. K., Sukla, L. B., Pattanaik, A., & Pradhan, D. (2020). Role of microalgae in treatment of acid mine drainage and recovery of valuable metals. *Materials Today: Proceedings*, *30*, 346-350.
51. Sargın, İ., Arslan, G., & Kaya, M. (2016). The efficiency of chitosan–algal biomass composite microbeads at heavy metal removal. *Reactive and Functional Polymers*, *98*, 38-47.
52. Sarojini, G., Babu, S. V., Rajamohan, N., Kumar, P. S., & Rajasimman, M. (2021). Surface-modified polymer-magnetic-algae nanocomposite for the removal of chromium-equilibrium and mechanism studies. *Environmental Research*, *201*, 111626.

53. Shamim, S. (2018). Biosorption of heavy metals. *Biosorption*, *2*, 21-49.
54. Shanab, S., Essa, A., & Shalaby, E. (2012). Bioremoval capacity of three heavy metals by some microalgae species (Egyptian Isolates). *Plant signaling & behavior*, *7*(3), 392-399.
55. Shen, L., Wang, J., Li, Z., Fan, L., Chen, R., Wu, X., ... & Zeng, W. (2020). A high-efficiency Fe2O3@ Microalgae composite for heavy metal removal from aqueous solution. *Journal of Water Process Engineering*, *33*, 101026.
56. Shokri Khoubestani, R., Mirghaffari, N., & Farhadian, O. (2015). Removal of three and hexavalent chromium from aqueous solutions using microalgae biomass-derived biosorbent. *Environmental Progress & Sustainable Energy*, *34*(4), 949-956.
57. Soeprobowati, T. R., & Hariyati, R. (2012, October). The potential use of microalgae for heavy metals remediation. In *Proceeding The 2nd International Seminar on New Paradigm and Innovation on Natural Sciences and Its Application, Diponegoro University, Semarang Indonesia* (Vol. 3, pp. 72-87).
58. Tripathi, S., & Poluri, K. M. (2021). Metallothionein-and phytochelatin-assisted mechanism of heavy metal detoxification in microalgae. *Approaches to the Remediation of Inorganic Pollutants*, 323-344.
59. UEPA, U. (2009). National primary drinking water guidelines. *Epa 816-F-09-004.*, *1*, 7. https://www.epa.gov/sites/default/files/2016-06/documents/npwdr_complete_table.pdf (accessed on 16/07/2021).
60. Wang, Z., Xia, L., Song, S., Farías, M. E., Li, Y., & Tang, C. (2021). Cadmium removal from diluted wastewater by using high-phosphorus-culture modified microalgae. *Chemical Physics Letters*, *771*, 138561.
61. Xia, L., Li, H., & Song, S. (2016). Cell surface characterization of some oleaginous green algae. *Journal of Applied Phycology*, *28*, 2323-2332.
62. Yang, J., Cao, J., Xing, G., & Yuan, H. (2015). Lipid production combined with biosorption and bioaccumulation of cadmium, copper, manganese, and zinc by oleaginous microalgae Chlorella minutissima UTEX2341. *Bioresource technology*, *175*, 537-544.
63. Yang, X., Zhao, Z., Yu, Y., Shimizu, K., Zhang, Z., Lei, Z., & Lee, D. J. (2020). Enhanced biosorption of Cr (VI) from synthetic wastewater using algal-bacterial aerobic granular sludge: Batch experiments, kinetics, and mechanisms. *Separation and Purification Technology*, *251*, 117323.
64. Yang, X., Zhao, Z., Zhang, G., Hirayama, S., Van Nguyen, B., Lei, Z., ... & Zhang, Z. (2021). Insight into Cr (VI) biosorption onto algal-bacterial granular sludge: Cr (VI) bioreduction and its intracellular accumulation in addition to the effects of environmental factors. *Journal of Hazardous Materials*, *414*, 125479.
65. Yen, H. W., Chen, P. W., Hsu, C. Y., & Lee, L. (2017). The use of autotrophic Chlorella vulgaris in chromium (VI) reduction under different reduction conditions. *Journal of the Taiwan Institute of Chemical Engineers*, *74*, 1-6.
66. Zhang, S., Rensing, C., & Zhu, Y. G. (2014). Cyanobacteria-mediated arsenic redox dynamics are regulated by phosphate in aquatic environments. *Environmental Science & Technology*, *48*(2), 994-1000.
67. Zohoorian, H., Ahmadzadeh, H., Molazadeh, M., Shourian, M., & Lyon, S. (2020). Microalgal bioremediation of heavy metals and dyes. In *Handbook of algal science, technology, and Medicine* (pp. 659-674). Academic Press.