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REVIEW

Phytoremediation of heavy metals by microalgae: a mini review

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Abstract There are five heavy metals, namely arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb) and mercury (Hg), which are carcinogenic and pose health risks, even in trace amounts. Due to their several benefits, microalgae are increasingly being used in phytoremediation to remove heavy metals, due to their abundant availability, low cost, and excellent metal removal efficiency. Using microalgae as soil remediation and biosorption agents for these toxic heavy metals, this review paper aims to report recent advances and mechanisms. The use of non-living biomass as biosorbents and the tolerance and response of different microalgae strains to heavy metals are discussed along with their bioaccumulation capability. Additionally, microalgae are explored as a bioremediation method for heavy metals. Overall, this review provides useful insights that can be applied to the development of efficient and commercially viable technology for heavy metal bioremediation using microalgae.

Keywords Heavy metals . Absorption . Adsorption . Bioaccumulation . Bioremediation

Introduction

The term "heavy metals" generally refers to a metal or metalloid with a density greater than 5 grams per cubic centimeter, which includes cadmium (Cd), chromium (Cr), arsenic (As), copper (Cu), mercury (Hg), iron (Fe), lead (Pb), zinc (Zn) silver (Ag), and others. Combustion of fossil fuels, along with other industrial activities, involve them in large amounts, and thus they are released into the environment every day via wastewater and other means (Zakhama et al. 2011). In nature, heavy metals can persist due to their non-biodegradable nature, resulting in bio-accumulation in food chains, leading to severe health and environmental problems (Yang et al. 2015). These substances have carcinogenic, mutagenic, teratogenic, bioaccumulating properties, and are non-biodegradable. These elements, when introduced into the body, can cause a variety of symptoms, including headaches and arthralgia at the mild end, mental disorders, abnormal liver and kidney function, and even cancer (Kong et al. 2021; Long et al. 2021) . The US Environmental Protection Agency (EPA) has listed the maximum contaminant limits (MCL) for Hg, As, Cd, Cr, Pb, and other HMs, which are reported in Table 1 (Burakov et al. 2018). There is a possibility that even extremely low concen-

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trations of these compounds could be toxic.

A variety of microorganisms such as microalgae, fungi, yeasts, and bacteria have been widely used for bioremediation of heavy metals due to their environmental friendliness and cost-effectiveness, especially at low heavy metal concentrations. Extreme environmental conditions may be a greater challenge for some microorganisms than for others. For example, microalgae are highly photosynthetically efficient and have a simple structure, so they can grow well in conditions of high salinity, heavy metals, nutrient stress extreme heat. Since microalgae have a high binding affinity, a large surface area, and an abundance of binding sites, they are increasingly used for phytoremediation of toxic heavy metals (Cameron et al. 2018). Additionally, microalgae biomass and living cells can both be used as biosorbents. Furthermore, bioremediation of heavy metals with microalgae not only offers outstanding removal capacity but is environmentally friendly, as well.

Microalgae have recently been identified as a key component of urban sustainable development. A study, conducted by the American Cleaning Institute, showed that microalgae have the potential to treat municipal wastewater (Balaji et al. 2016a; Lei et al. 2018). Nagarajan et al. (2020) presented the concept of circular bioeconomy for the recovery of carbon, nitrogen, phosphorus, and other nutrient elements from wastewater (Nagarajan et al. 2020). The process is robust and simple, with no toxicity restrictions; microalgae grow rapidly compared to higher plants, as well as are capable of producing value-added products like biofuels (Abinandan et al. 2019). Additionally, heavy metals induce oxidative stress in microalgae, which increases their lipid content. Notably, microalgae can be used to recover silver, thallium and gold ions (Jaafari and Yaghmaeian 2019).

This review discusses the recent advances in the bioremediation of five toxic heavy metals by microalgae, including cadmium, arsenic, mercury, lead, and chromium, due to their severe deteriorating effects on environmental ecology and human health.

Heavy metals in the aquatic system

Metallic elements can appear in a variety of chemical forms (species) in the aquatic environment. Sediment contains a variety of metals, including insoluble inorganic complexes, suspended particles, and organic colloids. A wide variety of metal species exist, including metal ions, inorganic complexes, and organic compounds. The equilibrium depends not only on the environment, but also on biota thriving in the water such as temperature, pH, and alkalinity. A trace metal's chemical form determines whether it is bioavailable as a nutrient (e.g., iron and zinc) or as a toxicant (e.g., cadmium and lead). Any process that accelerates the transformation of free metal ions into bound forms reduces the toxicity of free metal ions (Peterson 1982).

Current techniques for metals detoxification

Physicochemical approaches

Precipitation by pH adjustment, ion exchange, flocculation and/or organic adsorption and membrane filtration, are some of the most common physicochemical methods to remediate metal-contaminated waters. Target metals cannot be treated with these methods because they lack the specificity required. They are inefficient and expensive, especially if the wastewater concentration of metals is low. They are also difficult to operate and are expensive when used on a large scale *in situ*.

Table 1 Effects of the heavy metals and their maximum concentration limits

Heavy	MCL (mg/L)	Effects
As	0.01	Skin or the circulatory system
Cd	0.005	Kidney damage and osteoporosis
Cr	0.1	Allergic dermatitis, liver damage, and vomiting
Hg	0.002	Mental retardation, heart, brain, and kidney damage
Pb	0.015	Children: developmental delays in physical or mental development Adults: Hypertension and kidney disease

Biological approaches

Natural processes are used in biological approaches. Heavy metals are biogeochemically cycled by many microorganisms. Heavy metal ions in the environment are transformed by microalgae and other microorganisms (Haferburg and Kothe 2007). Growing cells and biodegradable products of different origins may release compounds that complex metal ions, thereby reducing their toxicity (Worms et al. 2006). Additionally, metal ions have been reported to bind to microalgae cell walls (Gupta and Rastogi 2008).The growth of microalgae involves a variety of metabolic processes, including photosynthesis, respiration, and nutrient uptake. All these factors influence the equilibrium between free metal ions and their bound forms. In metal-contaminated sites, microalgae have intracellular mechanisms that protect them from toxic effects (Seth et al. 2012).

Potential applications of microalgae in heavy metal bioremediation

Since 1990 the use of biological processes for removing, transforming, and detoxifying metals, including those describing microalgae, showed great promise (Mishra et al. 2011). For the removal of toxic heavy metals from contaminated sites, technologies based on naturally occurring biological processes may provide a number of advantages over physicochemical methods. Metal bioremediation can be carried out with many types of organisms (bacteria, algae, cyanobacteria, plants and fungi) including their dead biomass.

Microalgae mechanisms for removing heavy metals

Among the heavy metals consumed by microalgae, copper, boron, cobalt, manganese, iron, molybdenum, and zinc, can be listed. However, other heavy metals such as As, Pb, Cd, Cr, and Hg are toxic. The tolerance of some species of cyanobacteria *e.g Phormidium*, *Anabaena*, *Oscillatoria*, and *Spirogyra* to heavy metal pollution, makes them capable of growing in water contaminated by heavy metals (Balaji et al. 2016a). Additionally, microalgae can form complexes with wastewater pollutants that they bind actively through their reactive groups.

There are multiple strategies used by microalgae species to protect themselves against the toxic effects of heavy metals, including immobilizing heavy metals, regulating genes, excluding and chelating heavy metals, as well as antioxidants, which reduce heavy metals via redox reactions, or reducing enzymes (Gómez-Jacinto et al. 2015).

A strong affinity of microalgae towards metal compounds and ions is caused by the numerous binding sites on the membranes and walls of their cells. As demonstrated by theoretical analysis, various functional groups such as hydroxyl (OH-), carboxyl (-COOH), amino ($-NH_2$), and phosphate ($-PO_4$) groups accumulated on the surface of microalgae can produce negative charges, which play a crucial role in heavy metals adsorption. Furthermore, microalgae are composed primarily of proteins, polysaccharides, lipids, and membrane transporters are essential for coordinating microalgae with high levels of heavy metals (Li et al. 2020). Protein-heavy metal complexes can be formed by microalgae without affecting their own activity (Priatni et al. 2018). In addition to being separated within vacuoles, organometallic complexes help regulate the concentration of heavy metal ions within the cytoplasm, thereby mitigating their toxic effects (Balaji et al. 2016b). Additionally, heavy metals stimulate the production of phytochelatins (PCs), which are thiol-rich peptides and proteins that interact with heavy metals to minimize their stress (Gómez-Jacinto et al. 2015).

Catalase, ascorbate peroxidase, peroxidase, glutathione reductase and superoxide dismutase (SOD) are antioxidant enzymes that microalgae produce to neutralize free radicals released during adsorption of heavy metals. Furthermore, ascorbic acid (ASC), carotenoids, glutathione (GSH), cysteine, and proline are nonenzymatic antioxidants (Upadhyay et al. 2016). By breaking down the superoxide anion into oxygen molecules and hydrogen peroxide, SOD can be considered the first line of defense against it. In addition to hydrogen peroxide, catalase further degrades it into water and oxygen. Providing an indication of the synthesis of different antioxidants, cysteine is a precursor to PCs, GSH, metallothioneins, and other sulfur-containing compounds. Microalgae synthesize two endogenous antioxidants, GSH and ASC, which reduce free radicals and reactive oxygen species (ROS) (Leong and Chang 2020). Ascorbic acid-glutathione and metal-containing enzyme activity are also regulated by ASC in microalgal cells, making it an effective cell protector, dissipating excess excitation energy, and scavenging ROS, in addition to maintaining the equilibrium of ROS production and elimination. Moreover, microalgae secrete high levels of ASC as a hydrophilic redox buffer that protects cytosol and other cellular components from oxidative stress. In contrast, high levels of GSH provide microalgae with tolerance, scavenge free radicals, and facilitate PC and ASC synthesis as well as restore antioxidant substrate.

A two-stage mechanism accomplishes the removal of heavy metals by microalgae. In the first stage, the molecules are passively absorbed extracellularly (biosorption), while in the second stage, they positively diffuse and accumulate intracellularly (bioaccumulation).

Bioremediation and biosorption of heavy metals

Arsenic (As)

Among one of the major toxic heavy metals that contaminate potable water in many countries, including Bangladesh, Argentina, Finland, Chile, India, China, Southeast Asia and the USA, arsenic (As) is classified as class A and category 1 by the United States Environmental Protection Agency (USEPA). Arsenic contamination is widespread due to human activities, including burning fossil fuels, mining, using salt, fertilizers, pesticides, electrolysis, sewage sludge, and manufacturing pigments, semiconductors, glass, and alloys. It causes skin, lung, bladder and kidney cancer even at low concentrations (0.1 g/mL), whereas higher concentrations can cause arsenical dermatitis, diabetes, arsenicosis, cardiovascular disease, immune changes, impairment of the central nervous system, infant morbidity, hyperkerotosis and liver damage (Li et al. 2019). Alternatively, arsenic damages cell organelles, DNA, lipids, and proteins in plants and microalgae (Upadhyay et al. 2016).

As a result of its oxidative state and chemical form, arsenic has different toxicity and physicochemical properties. Among the four oxidation states of arsenic, there are arsenate (As(V)), arsine (As(-III)) and arsenate (As(-III)). There are two oxidation states that occur most commonly for As: trivalent [As (III)] and pentavalent [As (V)](Zhu et al. 2018). Several forms of As (III) in aqueous solutions exist, namely H3AsO3, H2AsO3, H2AsO3-, H2AsO3- and AsO33-. As (V) varies from H3AsO4-, H2AsO4, HAsO42- to AsO43-(Arora et al. 2018). It is much more toxic to consume arsenic in its inorganic form than in its organic form. In this way, arsenic can be transformed into its organic form, including monomethylarsonate (MMA) and dimethylarsinate (DMA) (Wongrod et al. 2019). By oxidizing As (III) and forming complexes with phytochelatins and glutathione, microalgae reduce the toxicity of inorganic arsenic, reducing As(V), biotransformation into arsenolipids/arsenosugars or methylated arsenic species, and adsorbing and excreting it from the cells, microalgae reduce arsenic toxicity (Papry et al. 2019). Aldehyde and aliphatic functional groups, such as –OH, –NH, –CH, as well as amino functional groups, mainly oxidize As(III) outside the cell. Arsenic metabolism in the cell is initiated by rapid reduction of As(V) to As(III), followed by slow methylation first to MMAs, then to DMAs. Microalgae may also excrete reduced arsenic species in addition to methylated arsenic species.

Due to its similarity in outer electron configuration to arsenate [As(V)], phosphate is important for protein synthesis, genetic material regulation, and protein modification via phosphorylation in microalgae. It is important for speciation, bioaccumulation, and detoxification of arsenic in microalgae (Sun et al. 2015). It is possible for microalgae to take up arsenic via high affinity phosphate transporters (such as PIT or PST). PO43− limitations induce the synthesis of arsenic transporter, which speeds up arsenic uptake (Wang et al. 2014). The uptake of arsenic by microalgae such as *Chlorella sp*,*C. vulgaris*, *C. salina*, *Dunaliella salina*, *Scenedesmus obliquus* and *Chlamydomonas reinhardtii* were reported to significantly reduce as a result of increased phosphate concentrations (Wang et al. 2013).

Cadmium (Cd)

Cadmium is an invasive heavy metal which is highly toxic and is released into the environment during the incineration of waste, manufacturing of metals and alloys, ceramics, pigment production, fertilizer and pesticide manufacture. manufacture, nickel-cadmium batteries manufacture, plastic production, electroplating and mining (Abinandan et al. 2019). Since cadmium is carcinogenic and teratogenic, even trace amounts can cause serious damage to reproductive organs, kidneys, liver, and lungs. In particular, cancer, Parkinson's and Alzheimer's diseases, gastrointestinal disorders, amyotrophic lateral sclerosis, hypertension, kidney damage, kidney stones, peripheral neuropathy, osteoporosis, and respiratory insufficiency can be mentioned as the most common health problems caused by cadmium (Zhang et al. 2019).

Various strains of microalgae respond differently to cadmium stress. Additionally, Cd(II) reduces cell growth and chlorophyll content in microalgae such as *Chlamydomonas moewusii* and *Monoraphidium* sp. by producing phytochelatins, superoxide dismutase, catalase, and peroxidase. A maximum adsorption capacity of 35.65 mg/g was observed for *Chlorella minutissima* UTEX2341. Microalgal lipid content and productivity were both enhanced by 0.6 mM Cd(II) uptake by 42.1% and 2.17 fold, respectively. This was also a higher lipid productivity than many reported in the literature, of 249.36 mg/L per day (Yang et al. 2015). The lipid content of *Monoraphidium* sp. QLY-1 exposed to 80 m Cd(II) was 1.59 times higher than the control (52.78%) (Zhao et al. 2019). The growth rate of *Chlorella vulgaris* was improved by 13.4% and the total lipids by 51.5% when 0.02 µm of free Cd(II) was added (Chia et al. 2013). Two acid-tolerant microalgae, namely *Heterochlorella* sp. MAS3 and *Desmodesmus* sp. MAS1, originally isolated from non-acidophilic environment, were tested for their ability to withstand higher concentrations of cadmium, at an acidic pH of 3.5. The growth analysis, in terms of chlorophyll, revealed that strain MAS1 was tolerant even to 20 mg L^{-1} of Cd while strain MAS3 could withstand only up to 5 mg L^{-1}. When grown in the presence of 2 mg L^{-1}, a concentration which is 400-fold higher than that usually occurs in the environment, the microalgal strains accumulated >58% of Cd from culture medium at pH 3.5 (Abinandan et al. 2019).

A variety of strategies have been studied to improve cadmium biosorption, including genetic engineering, bio-immobilization, microalgal immobilization, and bio-pellets. In *Chlamydomonas reinhardtii*, gene manipulation led to overexpression of the metal tolerance protein, which caused an increase in Cd^{2+} tolerance and uptake, respectively, which was 2.29 and 3.06 fold greater than that in wild type C. *reinhardtii* (Ibuot et al. 2017). Nevertheless, the naturally occurring strains of *Parachlorella kessleri*, *Parachlorella hussii*, and *Chlorella luteoviridis* showed greater tolerance to heavy metals.

By co-incubating with selenium, fluorescent CdSe NPs were bio-fabricated using *Microcystis aeruginosa* and *Selenastrum capricornutum* cells as bio-templates. Microalgae reducing biomolecules like glutathione, NADPH, and NADPH dependent reductase are suspected of playing an important role in CdSe NP formation (Zhang et al. 2019). At a low Cd^{2+} concentration of 20 ppm, *chlorella* sp. immobilized in alginate beads demonstrated a biosorption rate of up to 60% of Cd(II) (Valdez et al. 2018). Furthermore, due to the enhanced surface potential of *Chlorella* sp water hyacinth biochar complex, the bioremediation of cadmium achieved 214.7 mg/g higher than both *Chlorella* sp. and biochar alone. It is believed that cadmium is effectively absorbed because of functional groups such as -OH, -NH, phosphoryl groups, phosphonothioate groups, as well as amide groups and alcoholic groups (Shen et al. 2017). Waterhyacinth leaf biochar pellets immobilized with *Chlorella* sp. achieved 92.5% Cd(II) removal efficiency while remaining viable up to 10 mg/L Cd(II) (Shen et al. 2018). Compared with 40% of microalgae alone, bio-pellets generated from C. *vulgaris* microalgae and *Apsergillus niger* fungi were more effective at removing Cd(II) at low levels. They also have advantages such as lower pH values and simplicity of processing (Bodin et al. 2017).

Using non-living microalgae cells, passive surface binding of cadmium, has also been investigated. The presence of large amounts of acidic functional groups such as carboxylic acid in *Parachlorella* sp., is reported to make it a better biosorbent for Cd(II) than *Nannochloropsis* sp., S*cenedesmus* sp., and *Spirulina* sp.(Dirbaz and Roosta 2018). It has been shown that intracellular cell wall components can also contribute to heavy metal binding in *Chlamydomonas* sp. TAI-03, *Chlorella* sp. CHA-01, and *Coelastrum* sp. PTE-15 with broken cell walls obtained by lipid extraction (Zheng et al. 2016). Comparing freeze-dried *Chlorella minutissima* UTEX2341 biomass with growing algae, it was found that freeze-dried biomass is a superior biosorbent (Yang et al. 2015). The airgel beads containing microalgae *Chlamydomonas angulosa* and *Nostoc* commune exhibited an adsorption efficiency of 63.1% at 50 ppm Cd^{2+} at pH 6 (Hwang et al. 2018). Literature generally reports that Cd^{2+} adsorption follows Langmuir isotherms, indicating that adsorption is predominantly an energy-constant surface reaction (Ofudje et al. 2023).

Chromium (Cr)

Chromium compounds are used in dyeing, painting, inks, pigments, leather tanning, electroplating, steel production, and wood preservation (Gupta and Rastogi 2008). The two most common and stable oxidation states of chromium are hexavalent Cr (VI) and trivalent Cr (III) (Shokri Khoubestani et al. 2015). Since Cr(VI) can penetrate cell membranes easily affecting their integrity, differently from Cr(III) which is almost impermeable to them, it results much more toxic, even at very low concentrations (Pradhan et al. 2019). Additionally, Cr(VI) is highly soluble in water and has strong oxidizing properties, which may damage genetic material and alter DNA synthesis. Cr(VI) can cause chronic bronchitis, skin and stomach cancer, epigastric pain, kidney problems, tissue neurosis, ulcers or irritations of the digestive system, liver damage, internal hemorrhage, emphysema, and DNA polymerase impairment.

Microalgae reduce and remove Cr(VI) in various ways based on their functional groups and the conditions under which they operate. In *Phaeodactylum tricornutum* and *Navicula pelliculosa*, chromium bioremediation is primarily mediated by Cr(VI) biosorption on extracellular polymeric substances (Hedayatkhah et al. 2018)commonly designated as Cr (VI. A majority of Cr(VI) is accumulated in microalgal cells in organelles, granules, and cytosolic heat stable peptides and proteins (Aharchaou et al. 2017). Chromium reductase also eliminates heavy metals from the body and absorbs and accumulates them inside cells. It has been reported that after 24 hours of contact time with 100 ppm Cr(VI), *Chlorella sorokiniana* achieve an extraction efficiency of 99.7% after tolerating 100 ppm Cr(VI) for three days (Husien et al. 2019). In laboratory conditions and in natural water containing chromium, *Navicula subminuscula* can tolerate high concentrations of Cr(VI) up to 10 mg/L and 4 mg/L, respectively. Up to 98% of the heavy metal can be removed by the microalgae in cultures that contain 20 mg/L Cr(VI) (Cherifi et al. 2017)contact time and initial ion concentrations. The results show a significant growth of Navicula subminuscula for Cr concentrations up to 10mg L-1. The growth rate decreases as a function of increasing concentrations of Cr(VI. A combination of 55 mol/m^{2}s light intensity and a 3 mM sodium nitrate concentration, resulted in superior tolerance of Cr(VI) up to 1 mg/L for *Phaeodactylum tricornutum* and *Navicula pelliculosa*, while simultaneously accumulating lipids (Hedayatkhah et al. 2018)commonly designated as Cr (VI. *Pediastrum duplex* and *pseudobeanemucicola* can tolerate Cr(VI) up to 1.936 and 0.224 g/L, respectively, with the former removing 71% of Cr from the solution (Dao et al. 2018).

In the case of Cr(VI) removal using living *Anabaena*, *Phormidium*, *Oscillatoria*, and *Spirogyra sp*., carboxylates, ester groups, and hydroxyl groups were responsible for metal ion binding on microalgal cell walls (Balaji et al. 2016b). With increasing chromium concentrations, heavy metal stress reduced cell division and exponential growth of microalgae. The accumulation of NADH and H$^+$, caused by decreasing electron transport activity, is also responsible for the reduction of the total protein content of microalgae with increasing heavy metal concentration. As a result of its improved biosorption and biomass growth efficiency, as well as its increased antioxidant activity, the microalgae *Oscillatoria* sp. has been reported as among the best candidates for phytoremediation. In order to remove Cr(III) and Cr(VI), non-living biomass of microalgae can be utilized as biosorbent (Balaji et al. 2016b).

Lead (Pb)

Lead has been reported in a wide range of applications e.g. paint, battery making, cosmetics, weaponry, and building materials. The presence of lead in the environment poses a serious threat to human health and aquatic life. Children with Pb^{2+} can suffer serious physiological and mental problems, dementia, kidney dysfunction, including anemia, brain damage, and reproduction abnormalities (Dao et al. 2018). Various biosorbents have been used to remove lead in the environment, including *Phormidium sp*, *Spirulina platensis*, *Rhizoclonium hookeri*, *Chlamydomonas reinhardtii*, *Chlorella* sp and *Chaetoceros* (Suganya et al. 2016) the microalga *Rhizoclonium hookeri.*

Das and co-workers investigated particle adsorption and flow rate in a semi-batch biosorption process (2016). Various functional groups were identified for the biosorption of Pb^{2+}, including acyl-amino, amides, amines, carbonyls, carboxyls, hydroxyls, phenols, and phosphates. As pH decreases, lead ion adsorption decreases significantly due to electrostatic repulsion and competition with H$^+$ for binding sites due to high positive charge density on binding sites. Due to an increase in species such as Pb^{2+} and Pb(OH)$^+$ at pH 5-6, the biosorbent has the highest efficiency in removing Pb^{2+}.

At pH > 6, $Pb(OH)^2$ tends to precipitate (Akhtar et al. 2004) Chlorella sorokiniana, was immobilized on loofa (Luffa cylindrica. Microalgae biomass adsorbs Pb^{2+} spontaneously, endothermically, and chemically upon exposure to Pb^{2+} *via* its enthalpy change and enthalpy value. There is general agreement that microalgae biosorb Pb^{2+} using pseudo-second order kinetic models. It has been hypothesized that the rate-limiting step of chemical sorption occurs when Pb^{2+} and biomass share electrons and exchange ions.

Scenedesmus incrassatulus exposed to lead showed a decrease in peripheral cells of type "*incrassatulus*" and an increase in morphology types "obliquus". As a result, the peripheral morphology of *S. incrassatulus* can be used as a bio-indicator for lead pollution detection (Batsalova et al. 2017) mammalian cell lines are another type of test system that has been used to study the mechanisms of heavy metal toxicity. However, little is known about the sensitivity and potential application of different human cell lines for bio-monitoring of heavy metal contamination. Aim: To investigate the toxicity of increasing concentrations of cadmium, nickel and lead on the green microalga Scenedesmus incrassatulus and the human cell lines HeLa, A549, FL, and Caco-2. Materials and Methods: To evaluate the toxic effects of Cd, Ni, and Pb, two test systems were used: an algal culture of S. incrassatulus and four human cell lines. For the algal system, the growth of the algae and the features such as \”cell number in the coenobium/single cells\”, \”po-sition of the inner cells in the coenobium\”, and \”shape of the peripheral cell\”, were assessed. For the human cell cultures, the methyl-thiazol-tetrazolium (MTT. The chlorophyll fluorescence technology can also be utilized to detect lead toxicity in marine microalgae *Nitzchia closterium* (Gan et al. 2019) (Table 2).

Mercury (Hg)

In gaseous and aqueous form, mercury is primarily released by industrial activities such as mining, waste incineration, smelting, and coal combustion (Peng et al. 2017). Methylmercury (MeHg) is a potent neurotoxin that is linked to mercury. Consequently, mercury and its compounds pose significant risks to the environment.

In addition to its ability to penetrate the blood-brain barrier, mercury also alters the redox status of cells, interferes with essential metal uptake, and disrupts proteins and metal thiolate bonds in cells (Huang et al. 2006; Rezaee et al. 2006) Chlorella sp. DT, was transformed with the *Bacillus megaterium* strain MB1 merA gene, encoding mercuric reductase (Besides mental retardation, antibiotic resistance, and reproductive disturbances, they can also cause other adverse health issues. A "biomagnification" of mercury occurs throughout the aquatic food chain, similar to the bioaccumulation of hydrophobic organic compounds (Mason et al. 1996) HgCl2.

Acid reducible Hg^{2+} can be biotransformed into elemental Hg^0 and metacinnabar, which are varying levels of the chemical compounds (Kelly et al. 2007). By both biological and non-biological volatilization, Hg^0 is removed from Hg^{2+} following enzymatic reduction to Hg^0 by mercuric reductase. Hg^{2+} that remains unreduced is converted into β-HgS.

A large percentage of Hg^0 volatilization occurs rapidly, within 20 min to a few hours, as shown in selected microalgae as *Chlorella fusca*, *Selenastrum minutum*, the diatom *Navicula pellicosa*, and temperature-sensitive *Galdieria sulphuraria*. There is a difference between non-biological and biological volatilization of mercury. When illuminated by light, non-biological volatilization occurs, whereas biological volatilization occurs depending on the amount of metal and the density of the cells (Devars et al. 2000).

Dimethylfumarate-pretreated microalgae showed enhanced volatilization (Kelly et al. 2007). *Chlorella sp.* DT expresses *Bacillus megaterium* B1 mercuric reductase (MerA). Hg(II) removal ability in transgenic strains was improved up to two fold over controls and levels of oxidative stress were reduced (Huang et al. 2006). The removal of Hg(II) can also be accomplished by thiol chelation and bio-methylation to methylmercury (MeHg). However, mercury accumulation does not induce phytochelatin synthesis to the same degree as other heavy metals (Devars et al. 2000). Hg complexation with binding ligands is influenced by growth photoperiods. At longer light exposure periods, Hg-binding ligands are more homologous and aromatic in nature, while at darker growth conditions, Hg-ligand complexes are smaller and more aliphatic (Mangal et al. 2019).

Chlorella vulgaris has been pretreated for Hg^{2+} removal using polyelectrolyte self-assembly and biomimetic mineralization. Amorphous calcium phosphate mineral layers doped with sulfur atoms were deposited on the mineralized microalgae cells in addition to a polyelectrolyte coating.

Microalgae can grow well in high mercury concentrations of up to 100 g/L without obvious reduction in lipid yield because of the mineral layer protecting them from heavy metal poisoning. The optimal pH of C. *vulgaris* is 5.5, because Hg^{2+} competes with H^+ for binding sites at lower pHs, while $(Hg)OH^2$ complex formation at higher pHs significantly inhibits Hg^{2+} binding to organic functional groups. As a result of the higher degree of crystallinity on the mineralized layer and improved loading of S atoms, biomimetic mineralized C. *vulgaris* exhibits enhanced adsorption efficiency at pH 7, increasing as high as 94.7% (Peng et al. 2017).

Water treatment systems, utilizing membrane bioreactors, integrate membrane filtration with activated sludge for high efficiency. The bioremediation of Hg^{2+} from synthetic dental wastewater was achieved with the use of a dynamic membrane formed with *C. vulgaris* powder in a dynamic membrane bioreactor. In addition to promoting removal yields, fouling was reduced, and membrane recovery was less expensive. Hg^{2+} removal efficiency was higher in dynamic membrane bioreactors than control membrane bioreactors

Table 2 Biosorption performance of arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), and mercury (Hg) by different microalgae strains.

Microalgae strain	Temp (°C)	Optimal pH	Initial metal conc. (mg/L)	Biomass conc. (g/L)	Time (min)	Max. sorption (mg/g)	Removal efficiency (%)	Reference
The capacity of different microalgae strains to biosorb arsenic								
Maugeotia genuflexa	20	6	10	4	60	2.4	96	(Sarı et al. 2011)
Spirulina sp.	35	6	7276	12	240	365	60.2	(Doshi et al. 2000)
Ulothrix cylindricum	20	6	10	4	60	2.45	98	(Tuzen et al. 2009)
Chlamydomonas reinhardtii	–	9.5	12	1	180	4.63	38.6	(Saavedra et al. 2018)
Scenedesmus almeriensis	–	9.5	12	1	180	5.0	41.7	
The capacity of different microalgae strains to biosorb cadmium								
Chlamydomonas sp.	30	7.5	–	1	60	23.3	–	(Zheng et al. 2016)
Chlorella sp.	30	7.5	–	1	60	25.5	–	
Coelastrum sp.	30	7.5	–	1	60	32.8	–	
Chlorella minutissima	28	6	–	4	20	303	–	(Yang et al. 2015)
Parachlorella sp.	35	7	100	1	–	96.2	–	(Dirbaz and Roosta 2018)
Scenedesmus-24	–	6	200	1.5	–	48.4	60.5	(Jena et al. 2015)
The capacity of different microalgae strains to biosorb chromium								
Spirulina platensis	60	1	500	–	90	59.6	–	(Nithya et al. 2019)
Scenedesmus quadricauda	25	6 for Cr(III)	100	2	120	–	98.3 for Cr(III)	(Shokri Khoubestani et al. 2015)
Spirulina platensis	25	1.5	250	1	600	148.64	59.5	(Gokhale et al. 2008)
Chlorella vulgaris	25	1.5	250	1	600	140	56	(Sibi 2016)
Rhizoclonium hookeri	–	2	1000	1	45	67.3	6.7	(Kayalvizhi et al. 2015)
The capacity of different microalgae strains to biosorb lead								
Chaetoceros sp.	25	6	20	1.5	180	8	60	(Molazadeh et al. 2015)
Phormidium sp.	25	5	10	4	40	2.305	92.2	(Das et al. 2016)
Rhizoclonium hookeri	40	4.5	–	–	–	81.7	–	(Suganya et al. 2016)
The capacity of different microalgae strains to biosorb mercury								
Chlamydomonas reinhardtii	25	6	500	–	120	106.6	–	(Bayramoğlu et al. 2006)
Chlorella sp.	30	–	8	0.3	120	3.33	12.5	(Huang et al. 2006)
Chlorella vulgaris	20	5	48	2	120	17.49	72.9	(Solisio et al. 2019)
Scenedesmus obtusus XJ-15	25	5	20	0.125	180	–	–	(Huang et al. 2019)
Spirogyrasp.	4	4	1	3	30	0.253	76	(Rezaee et al. 2006)

at 300-800 ppb concentrations (Fard and Mehrnia 2017).

In order to protect aquatic ecosystems, heavy metal pollutants must be monitored rapidly and sensitively. *Halamphoraveneta's* frustule deforms and grows when exposed to mercury, along with significant increases in SOD gene expression and activity. As a result, *Halamphoraveneta* can be evaluated for mercury toxicological effects on aquatic ecosystems based on changes in SOD gene expression levels and activity, frustule morphology, and chlorophyll a concentration (Mu et al. 2017) (Table 2).

Challenges

There are many benefits to using microalgae for heavy metal bioremediation; however, they still face a number of challenges, such as contamination by other nutrient variability, microorganisms, high TSS and turbidity content, harvesting microalgae biomass, and downstream processing. In Table 3, the major challenges of removing heavy metals using microalgae-based biosorbents, as well as some of the strategies that might be used to overcome these challenges, are summarized. Microalgae showed a high potential for heavy metal removal when pretreatment steps are taken or when other technologies are integrated with them. Further, improving heavy metal remediation efficiency and reducing operating costs will require the isolation and development of new microalgal strains. The information presented in Table 3 indicates that microalgae can potentially remove heavy metals more effectively than conventional methods. There are still several areas for improvement, including the isolation of more powerful microalgal strains and the development of better treatment processes, to further increase the efficacy of heavy metal remediation and to reduce the operating costs.

Future prospects

On the basis of the considerations discussed in this contribution, microalgae may be a promising resource for heavy metal bioremediation given their multiple advantages.

The first step in bioremediation of heavy metals wastewater is to screen and select the appropriate microalgal strains. Additionally, it is beneficial for microalgae to have high tolerance for heavy metal pollutants and fast and stable growth. Among these are: (i) the ability to accumulate lipids and other valuable co-products, (ii) the ability to sequester CO_2 and low nutrient requirements, (iii) resistance to grazers and robustness against the presence of other microorganisms, and (iv) the ability to self flocculate for cell harvesting at a low cost.

Genetic, metabolic, and molecular engineering can, therefore, enhance the adaptive capability, specificity, and robustness of microalgae strains. In addition, research into the mechanisms underlying heavy metal biosorption and bioaccumulation by microalgae as well as the development of new equilibrium and kinetic models is crucial to better understanding this process. A number of techniques for the removal and recovery of heavy metals, including whole-cell immobilization, pelletization, and microalgal biofilms, have recently gained attention due to their potential industrial applications. Microalgae biomass can also be improved by using surface and chemical modification techniques, as well as integrating them with other heavy metal removal techniques.

Table 3 Heavy metal removal by microalgae: challenges and strategies

Challenges	Proposed strategies
Heavy metal wastewater consists of bacteria, fungi, and other microorganisms that compete for nutrients and may even dominate, due to their relatively faster growth rates.	* Treatment techniques (acidification, autoclaving, exposure to high levels of ammonia, chlorination by bleach, ozonation, filtration, and ultraviolet irradiation) * Utilization of waste nutrient-rich materials for nutrient supplementation * The high strength wastewater should be diluted with the low nutrient wastewater.
Variability in nutrients (such as excess or deficiency in carbon, nitrogen, and phosphorus).	* Choose microalgae strains that can adapt to your wastewater's specific conditions. * Microalgae species isolated from heavy metal wastewater are screened and isolated.
Water containing TSS and high turbidity may have a negative impact on microalgae growth.	* Methods of pretreatment (such as the use of flocculants and gravity sedimentation). * Increase light exposure by creating turbulence, such as in raceway ponds.
Harvesting of microalgae biomass	* Incorporating several harvesting techniques and integrating them with other technologies (e.g., membrane technology and electrokinetics). * The development of nanoparticle separators, magnetic agents' coagulants, and novel organic flocculants.
Presence of heavy metal remaining in microalgal cells might interfere with downstream processing of valuable product	* Various strategies can be utilized, including immobilization, microalgal biofilms, palletisation, and co-cultivation with flocculating microorganisms.

Despite the small size of microalgae, harvesting their biomass has always been a challenge. To reduce overall operating costs, it is essential to develop new techniques and improve traditional harvesting technologies. It has been reported that, at certain concentrations, some heavy metals, including aluminum, as, metallic nanoparticles, cadmium, copper, lanthanide elements, Pb, and others, have a positive effect on microalgae growth, even though they normally inhibit the growth of microalgae(Miazek et al. 2015). In the same way, certain heavy metals also contributed to the accumulation of lipids (like Cd, As, Ni, Cu), pigments (like As, Cd, Cu, Fe, Ni and Te), phytochelatins (like Cd, As, Pb, Cu), exopolymers (like Ag, Cd, Co, Cu), and phytohormones (like Pb, Cu, Cd) due to their inducing effects.

Suitable and quality biodiesel can be produced by modifying microalgal fatty acid compositions under heavy metal stress (Miazek et al. 2015). However, these valuable products may be adversely affected by heavy metals. For the simultaneous production of value-added products and heavy metal bioremediation to be successful, there is a need for further research into the upstream cultivation process and the downstream purification process.

Conclusion

Microalgae bioremediation of aqueous effluents, containing various heavy metals, has gained popularity recently due to its high efficiency, minimal secondary (chemical or biological) waste, and low cost. These biosorbents have been reported to interact with large volumes of aqueous effluents to remove heavy metals with relatively low metal ion concentrations (10-100 mg L^{-1}). The use of microalgae as biosorbents is highly dependent upon the variety of functional groups present on the biosorbent surface and their uniform distribution on the surface of these organisms as well as their reduced preference for alkali and alkali-earth metal ions in comparison to heavy metals. Microalgae can grow both in salted and fresh water in many regions. As a chemical substrate, microalgae serve as biological substrates with a resilient structure, with functional groups within biomass skeletons function as binding sites for heavy metals in aqueous solutions. The following factors should be considered when designing a bioremediation process to remove heavy metals from water by biosorption on microalgae:

(1) Several factors, which contribute to the efficiency of the biosorption process, such as particle size, growth conditions, cell pretreatment, as well as the retention process (aqueous solution pH, biosorbent dose, work methodology, temperature, height of biosorbent bed, aqueous solution flow rate, concentration of heavy metals, etc.), must be optimized.

(2) To model the biosorption of heavy metals on microalgae, several well-known equilibrium and kinetic models are available. These models provide useful information about the mechanism by which heavy metals are assimilated into the microalgae. Initially, experimentation must be conducted under batch conditions to obtain some basic information. Afterwards, it is necessary to test the potential applicability of biosorption systems in continuous-flow conditions to assess their potential for large-scale use. Biosorption systems are evaluated for performance and predicted based on equilibrium as well as dynamic studies, which ultimately lead to equipment sizing.

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