MIKAILALSYS
Journal of Multidisciplinary Sciences

e-ISSN : 2987-2286
p-ISSN : 2987-3924

Index : Dimensions, Scilit, Crossref, Garuda, Semantic, Lens,Google Scholar,Base, etc

https://doi.org/10.58578/mikailalsys.v2i1.2660

A REVIEW OF PHYCOREMEDIATION OF HEAVY METALS IN INDUSTRIAL WASTE WATER

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Article Info:

Submitted:	Revised:	Accepted:	Published:
Jan 15, 2024	Jan 20, 2024	Jan 25, 2024	Jan 28, 2024

Abstract

Environmental Pollutants such as heavy metals poised serious threat to biological systems. Heavy metals which could be natural or anthropogenic sourced are non-degradable pollutants and must be eliminated or reduced to certain quantity that is analytically presumed to be a safe limit before discharging into the environment to avoid injurious effects on biological systems. This review work showed the abilities of microalgae to biologically remove heavy metals from waste water through the process known as 'Phycoremediation'. Phycoremediation of heavy metals with the help of microalgae takes place in two stages: Biosorption and Bioaccumulation. Microalgae such as Nannochloropsis oculata, Scenedesmus sp, Scenedesmus obtusus, Gracilaria corticata, Sarcodia suidae and Spirulina sp has been proven to phycoremediate; Pb, Cr, Hg, As, Cd, and Cu respectively. Therefore, this

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review work has shown that the microalgae are efficient biological vectors for heavy metal uptake.

Keywords: Microalgae, Phycoremediation, Heavy metals, Bioremediation, Waste water

Introduction

The curiosity of humans and their activities haves resulted to several challenges such as water pollution, food insecurity and unsustainable environment. Globally, by 2050 water demands for agriculture, industry, and municipalities are expected to rise by 20–30% (Boretti and Rosa, 2019). One effect of this rise is the generation of more waste water in more ways and amounts, all of which are contaminated with a variety of chemicals in various concentrations. Heavy metal deposition has been caused by the usage of many tons of insecticides and herbicides on crops each year (Bockstaller *et al*., 2009), and by the means of biosorption and bioaccumulation, levels of heavy metals ions may rise throughout the food chain and ecosystem (Mehmood *et al*., 2019), become toxic to health and exert severe and acute effects on animals, plants and humans (Umer *et al*., 2018). Also wastes from different industries such as steel, textile, mining etc., enters aquatic environments through sewages, ballast water in shipping, runoff, leaching, and other activities, (Sononeet *et al*., 2020). Industrial wastes made up of inorganic contaminants that include different groups of heavy metals such as mercury (Hg), cadmium (Cd), cobalt (Co) copper (Cu), zinc (Zn), lead (Pb), nickel (Ni), chromium (Cr) and Arsenic (As) (Naskar *et al*., 2018) have devastating impact on water quality and consequently on biota and human health (Mosleh *et al.,* 2012).

Heavy metals are elements with atomic weights ranging from (63.5g/mol - 200.6g/mol) and basic gravity greater than 5.0 are non-degradable and can become dangerous due to their toxicity, and tendency to accumulate in living bodies in higher levels (Bando *et al*., 2019; Fatima *et al*., 2020; Bando *et al*., 2023). In recent years, pollution by heavy metals has become a significant concern. Due of their rapid mobility, lack of degradation, and high potential to accumulate within all living things, heavy metals can pose a threat to ecosystems and public health. Additionally, despite having low concentrations in the environment, they can participate in biological processes that damage essential functions in

cells, tissues, and organs, thereby promoting disease (Topcuoglu *et al.*, 2003; Mosleh, 2013). However, some of these metals—such as zinc, copper, manganese, nickel, and cobalt are crucial micronutrients for plant growth at low concentrations, while others such as Cd, Pb, and Hg might have harmful biological effects (Gaur and Adholeya, 2005). Mercury, cadmium, copper, zinc, lead, and nickel, among other detrimental heavy metals, are the most common contaminants in both marine and freshwater (Mosleh, *et al.*, 2014).

The conventional treatment methods for extracting biologically polluted heavy metals or effluents of wastewater include ions exchange, reduction of contaminants, chemical precipitation, membrane filtration, nanotechnology, electrochemical removal and advanced oxidation (Chalivendra, 2014). Furthermore, there are several biological processes that use inactive or active, free or immobilized cells of algae or plant tissues, as cells have carbohydrates and polypeptides in their walls with, aldehydes, hydroxyl, amines, ketones, carboxyl groups and phosphates responsible for adsorption and chelation (Wang, 2009). Nevertheless, considering these conventional techniques are limited due to significant disadvantages, such as low selectivity, incomplete removal, high energy consumption, high cost or high toxic waste generation. Therefore, the world is constantly in need to find safe, low-cost and more effective alternative techniques for removing heavy metals from contaminated water.

Bioremediation is an environmentally friendly process that utilizes the natural ability of organisms like fungi, bacteria and algae to detoxify inorganic and organic contaminants from industrial effluent (Bharagava *et al.*, 2018; Abuchi *et al.*, 2022). In the bioremediation process, pollutants are converted into some inorganic substances, water and carbon dioxide, and thus results in neutralization (Saxena *et al.*, 2020). Bioavailability of pollutants, which has a significant impact on the metabolic capacity of microorganisms to digest and modify contaminants, is a key component in bioremediation. Due to its numerous benefits, including cheap cost and high effectiveness, biosorption is thought to be a powerful alternative method for removing heavy metals from wastewater (Han *et al.*, 2020). Microalgae are ideally suited for analysing water quality and have been demonstrated to be reliable bio indicators because they have fast reproduction rates, very sensitive responses to chemical changes, eutrophication and pollution, and receive a wide variety of typically specific species (Larson and Passy, 2012; Mofeed, 2020).

Microalgae are unicellular organisms that consist of both prokaryotic (Cyanophyceae) or eukaryotic (Chlorophyta) organisms and they can grow rapidly in moist environments such as fresh water, waste water, and the marine environment. Among various microalgae, a few selected species such as *Scenedesmus, Chlorella*, etc. have the capacity to survive in the most extreme environments (e.g., high temperature and high CO2) (Maity *et al.*, 2014). The ability of microalgae to remove and detoxify heavy metals is the result of several adaptive mechanisms established over centuries of evolution (Monteiro *et al.*, 2012). Harnessing this natural power in the treatment of heavy metals contaminated water seems to be a promising strategy.

Microalga contains multifunctional macromolecules such as lipids, proteins and carbohydrates, which offer on its surface different negatively, charged functional groups such as amino, hydroxyl, carboxyl, sulfhydryl, sulfate, phosphate, phenol etc. (Javanbakht *et al.*, 2014). These negatively charged groups allow the binding of ions from the surrounding environment, making the outer layer of the cell wall as the first participator in the removal of heavy metals (Leong and Chang, 2020; Singh *et al.*, 2021). Therefore, knowing the structure, composition, and properties of the cell wall is essential when studying biosorption mechanisms (Podder and Majumder, 2017). The adsorption mediated by exopolysaccharide is an energy-independent process that occurs via the electrostatic interface between heavy metal cations and the negatively charged functional groups of exopolysaccharides (Kim *et al.*, 1996). Functional groups found in active components for heavy metal uptake include amine, hydroxyl, carbonyl, sulfhydryl, and phosphonates. Metals such as lead (II), chromium (IV), and copper (II) can be accumulated via carboxyl and amino groups with the help of displacement of protons (Dobrowolski *et al.*, 2017; Wang *et al.*, 2017). Carboxyl groups associated with extracellular polymeric substances assist in binding heavy metals and blocking of the carbonyl group resulted in decrease in metal binding (Yue *et al.*, 2015). The amine group could be positively charged during protonation and forms metal complexes that are negatively charged. Mostly the amine group is found associated with chitin and polysaccharide which contribute metal adsorption (Liu *et al.*, 2017). It has been shown that phosphoryl group is another binding site for the detoxification of heavy metals (Schultze-Lam *et al.*, 2018).

Sources of heavy metals

Heavy metals are one of the most dangerous pollutants responsible for global environmental damage (Onyekwere *et al*., 2021; David *et al*, 2022; Bando *et al*., 2023). Industries making batteries, automobiles, mining, smelting of ores, agricultural materials like fertilizers and pesticides release heavy metals like arsenic, lead, chromium, cadmium, mercury, nickel, silver, and uranium, etc., into the environment. These are non-biodegradable in nature and have a hazardous impact on the whole ecosystem. Heavy metals are indiscriminately used for anthropogenic purposes which alter the bio-geochemical cycles. The presence of the non-permissible amount of heavy metals in the soil, air and water cause harmful effect on the ecological diversity of the earth (Onyekwere *et al*., 2021). Their accumulation into the environment leads to biomagnification (Ali *et al*., 2019). Heavy metals also tend to cause respiratory, renal, mental and cardiovascular disorders and leads to cancer (Bando *et al*., 2019). Heavy metals can be sourced naturally or through man-made activities;

1. Natural Sources

Chromium: Chromite ore (FeCr2O4) is a mineral found in mafic and ultramafic rocks and is a complex of various quantities of magnesium, iron, aluminum, and chromium. It is composed of the two most stable oxidation states of chromium, i.e., Cr(III) and Cr(VI) (Al-Battashi *et al*.,2016).The naturally released Cr (III) ion can most probably oxidize into a highly detrimental form (i.e., Cr (VI)) through microbial intervention and geochemical processes (Tumolo *et al*., 2020). Also, geologic parent materials or rock outcroppings and volcanoes are the most important natural supply of chromium pollution in the environment (Mishra *et al*., 2015). Cr is also present in all sorts of environmental elements, including air, water, and soil, of course, but in limited amounts (Jobby *et al*., 2018).

Lead: Lead is an abundant heavy metal in the Earth, about 14 parts per million by weight or 1 part per million by moles. It rarely occurs in pure form in nature. Lead is usually found in ores, mostly with copper, zinc and silver. The most common lead mineral is galena, which is lead sulfide (PbS). Other minerals include lead carbonate (PbCO3) and lead sulfate (PbSO4) (Yongsheng *et al*. 2011; Najeeb *et al*. 2017).

Arsenic: The main natural sources of Arsenic emissions into the atmosphere include volcanic activity, biovolatilization, and soil erosion from wind (Najeeb *et al*. 2017).

Mercury: Mercury (Hg) is a ubiquitous, very persistent element which can be found literally everywhere. It is a naturally occurring metal on earth, shiny silver-white, odorless liquid and becomes colourless and odourless gas on heating. It is highly toxic and exceedingly bioaccumulative in nature (Sutton *et al.* 2002). There is presence of adverse effects of mercury on marine environment and hence many latest research projects are directed towards the distribution of mercury in water environment.

Cadmium: Cadmium is a relatively rare element (0.2 mg/kg in the earth crust) and is not found in the pure state in the nature. It occurs mainly in association with the sulfide ores of zinc, lead and copper. Cadmium has only been produced commercially in the twentieth century (Bernard *et al.* 2008).

Copper: copper occurs combined in many minerals, such as chalcocite, chalcopyrite, bornite, cuprite, malachite, and azurite. It is found in the ashes of seaweeds, in many sea corals, in the human liver, and in many mollusks and arthropods (Yongsheng *et al.* 2011).

2. Anthropogenic Sources

Chromium: In addition to the natural processes already in place, numerous industrial operations release chromium into water bodies, posing a serious risk to aquatic and terrestrial life and raising concerns across the globe. As a result, chromium and its derivatives are used in numerous industrial processes for a variety of reasons. Among those manufacturing industries metallurgical, refractory, oil well drilling, metal plating, mining, textile, pulp, petroleum, chemical production, and leather consume chromium in varied quantity for different applications (Carolin *et al.*, 2017, Tripathi *et al.*, 2018). Among the most commonly existing oxidation states, Cr (VI) concentrations have risen significantly in both aquatic and terrestrial ecosystems over the past few decades. It derives mainly from dyeing, textiles, steel fertilizers, photography, electroplating, manufacturing, packaging, and leather tanning and finishing (Al-homaidan *et al.*, 2018).

Over all, the most stable species of chromium, Cr (III) and Cr (VI), are synthesized both naturally and anthropogenically. The earlier one is mainly produced naturally by rock weathering, volcanic activities, and other geologic process, which gets oxidized to highly detrimental Cr (VI) form by microorganisms in the environment. The hexavalent form

highly formed through anthropogenic process by human activities rather than natural process.

Arsenic: It is released through anthropogenic activities like mining, smelting, medical, chemical and agricultural wastes. It is present in fungicides, pesticides, and herbicides. It is used in the alloy manufacturing processes (Tripathi *et al.*, 2018). There are two forms of arsenic i.e. arsenate and arsenite which are easily taken up by plants and eventually enters the food chain.

Copper: There are many significant sources of copper that result from human activity. These comprise; burning of fossil fuels, particularly coal, which releases copper in both fly and bottom ash, industrial incinerators, used motor oils, city water treatment sludge, sewage, and sewage sludge and so on (Focardi *et al* 2013; David *et al*, 2022). The copper in copper plumbing dissolves slowly and adds copper to the water supply and waste stream, copper roofs leach copper when exposed to acidic rain, and brake pads of modern cars contain copper which wears away over the course of use. Copper has a long history of being used as a pesticide or fungicide, and is often released into the environment for that purpose (Tripathi *et al.*, 2018).

Cadmium: Cadmium is used as a stabilizer, or present in dyes, fertilizers; and batteries such as nickel-cadmium battery; It is also released in fuel combustion and act as a corrosive agent (Liu *et al.*, 2008). It is a non-essential heavy metal for humans. Industries like metal industries, electroplating, smelting, plastic, dyeing and oil refining industries release of cadmium into the environment. Processes in the agricultural sector including fertilizer and pesticide industries, semiconductor, welding, cigarette industries, and nuclear plants release cadmium into the environment (Liu *et al.*, 2008; Focardi *et al* 2013).

Mercury: The widespread use of mercury in industries like mining, smelting, coals, electronics, pesticides, and chemical etc., is the main reason behind its release into the environment (Ratnaike 2013). Mercury is added in agricultural land through manures, sludge, fungicides or fertilizers. Mercury is converted into the neurotoxin methyl mercury.

Lead: Lead is used in various industries like coal, batteries, gasoline, pipelines and pigment.

Toxicity of heavy metals

Chromium: The International Agency for Research on Cancer (IARC) of the World Health Organization (WHO) designated Cr(VI) compounds as group one human carcinogens with numerous complex modes of action based on epidemiological studies tying Cr(VI) to lung cancer (Seidler *et al.,* 2013). Human subjection to Cr(VI) can cause allergies, irritations, eczema, ulceration, nasal and skin irritations, eardrum perforation, respiratory tract disorders, and lung cancer (Focardi *et al* 2013). Cr (VI) exposure may open up to cytotoxicity, genotoxicity, and carcinogenicity of Cr(VI) containing compounds, DNA mutations, and chromosomal damage at different stages, as well as to oxidative protein changes (Yang *et al*., 2019). Moreover, taking in high concentrations of hexavalent chromium can cause nose ulcer, irritation to the lining of the nose, anaemia, irritation and ulcer in the small intestine and stomach, as well as other respiratory issues such as wheezing, coughing, nasal blockage, and facial erythema (Engwa *et al*., 2019). Chromium compounds most commonly found in the soil in the form of $HCrO_4^-$ and CrO_4^{2-} which get easily sucked up by plants and rapidly trickle down into the deep layers of soil, thereby endorsing surface and groundwater pollution (Elahi *et al*., 2020). Due to its relatively low solubility, Cr(III) is thought to be the least damaging of the many oxidation states and prevents its leaching into groundwater or absorption by plants, whereas Cr(VI) has been proven to significantly injure living cells (Cervantes *et al*., 2001). Although some crops are not affected by reduced levels of Cr (3.810−4 μM) (Shanker *et al.,* 2005), chromium compounds are highly toxic to most plants and are detrimental to their production and growth.

Mercury: Mercury causes nervous disorders, gene expression alterations, autoimmune diseases, depression, gingivitis, irritation in eyes, mental disorders, reproductive disorders, antibiotic resistance, chest pain and interstitial pneumonitis which ultimately leads to death (Liu *et al*., 2008; Bando *et al*., 2019). It affects kidney, induces restlessness, and anxiety in exposed human population. Aquatic plants accumulate mercury inside them. Organic mercury is 200 times more lethal than inorganic mercury as it is formed by the combination of mercury and carbon resulting in the formation of compounds such as methylmercury which is neurotoxic in nature. In microbes, it causes protein denaturation, disruption in outer layer of cells; inhibit enzymatic activity as well as transcription (Ratnaike 2013). In plants, it alters the permeability of cell envelop and causes abnormal germination (Liu *et al*., 2008). It decreases chlorophyll synthesis, photosynthesis, anti-oxidant defence mechanisms

and transpiration rates. It disturbs the balances essential ions like potassium (K), magnesium (Mg), manganese (Mn) and iron (Fe) concentration (Yoshida *et al*., 2009).

Arsenic: Toxicity and health impact related to significant exposure to As are dependent on the time and dose of exposure. Acute toxicity and chronic toxicity are two different categories for As-induced toxicity (Ratnaike 2013). Acute toxicity involves negative effects in an organism through a single or short-term exposure. Ingestion of inorganic As (60–120mg as As_2O_3) would result in mild toxicity characterized by vomiting, abdominal pain, bloody diarrhoea, which lead to dehydration, convulsion, coma, and death (Ravenscroft *et al*., 2009) Arsenic is recognised to have mutagenic and genotoxic impacts on humans, and it has been related with optimum risk of skin, lung, liver and other cancers, type 2 diabetes, and reproductive and developmental problems (Liu *et al*., 2008). Due to Arsenite (III)-induced toxicity, affinity for sulfhydryl groups and binding with reduced cysteins in proteins causes its conformation shift and subsequent functional inactivity (Shen *et al.,* 2013). Thus, under the arsenite stress, various cellular functions such as the Krebs cycle, cellular glucose absorption, gluconeogenesis, fatty acid oxidation, etc. become disrupted (Shen *et al.,* 2013).

Since both the phosphate and the pentavalent forms of inorganic arsenic, or arsenate; belong to the same group in the periodic table of elements and have related structures, their molecules are comparable. Thus, in some biological reactions, these can resemble and replace phosphate. In a lab setting, arsenate easily combines with glucose and gluconate to generate the molecules glucose-6-arsenate and 6-arsenogluconate, which are both related to the corresponding phosphate products (glucose-6-phosphate and 6-phosphogluconate). The phosphate analogue enzyme, glucose-6-phosphate dehydrogenase, can also act as a substrate for the glucose arsenate, which then inhibits the hexokinase (Lagunas, 1980). Arsenate can also struggle for the phosphate anion transporter and sodium pump and thus lower its uptake (Kenney and Kaplan, 1988). The toxic impact of As in humans depends on age, gender, and state of health as well as mode of feeding of the individual (Mathew and Beeregowda, 2014). Several reports have illustrated negative impacts of arsenic exposure on memory-cognitive development, intelligence-adverse pregnancy outcomes, and infant mortality, with impacts on child health (Quanash *et al.,* 2015). The oxidative stress, altered DNA methylation, altered DNA repair, mitochondrial damage, cell proliferation, tumour promotion, and co-carcinogenesis caused by arsenic species that

accumulate in the skin, lungs, kidney, liver, etc. result in serious disorders (Zhuang *et al.,* 2013; Abdul *et al.,* 2015).

Cadmium: Cadmium enters the food chain and then to the gastrointestinal tract, affecting organs like heart, kidney, liver, and reproductive organs. It is considered to be teratogenic and carcinogenic. Cadmium is inhaled by the humans and it affects the chest and lungs (Bando *et al*., 2019). It causes nausea, vomiting, cough and other complications. It affects the growth, morphology as well as cellular metabolism, photosynthesis in plants and microbes and sometimes leads to death (Yoshida *et al*., 2009).

Lead: Lead toxicity causes ionic and oxidative stress mechanisms in living cells. The oxidative stress in living cells occurs due to the imbalance in the production of free radicals and creation of antioxidants to detoxify the reactive intermediates. Presence of antioxidants like glutathione, in the living cells protects them from free radicals (e.g. H_2O_2). The reactive oxygen species (ROS) level increases and the level of antioxidants decrease due to the influence of lead (Scheiber *et al*., 2013).

The ionic mechanism of lead toxicity occurs principally with the ability of lead metal ions to replace other bivalent cations (Ca2+, Mg2+, Fe2+) and monovalent cations (Na+) which intern ultimately disturbs the biological metabolism of the living cells. This ionic lead toxicity also causes considerable changes in cell adhesion, intra- and inter-cellular signaling, protein folding, maturation, apoptosis, ionic transportation, enzyme regulation, and release of neurotransmitters (Flora *et al.* 2008). Ionic lead toxicity may also substitute calcium even in picomolar concentrations which affects protein kinase C which in turn influence the neural excitation and memory storage (Flora *et al.* 2008).

Copper: Copper compounds are hazardous through inhalation, eating, drinking, and dermal subjection to environmental conditions that contain copper (Anant *et al*., 2018). Menkes disease and Wilson's disease, two serious disorders in humans that cause the loss of important organs, alter lipid peroxidation in liver mitochondria, and lower amounts of the antioxidant vitamin E in the liver and blood, are linked to Cu toxicity (Gaetke *et al*., 2014).

Chronic Cu toxicity triggers oxidative damage, neurodegenerative diseases like Alzheimer's, Parkinson's, and Huntington's, hepatic disorder (liver cirrhosis), and other disease conditions, such as Cu-protein interaction in the nervous system, temporal and spatial distribution of Cu in hepatocytes, activation of acidic sphingomyelinase and release of

ceramide, alpha-synuclein aggregation, as well as lipid metabolism, genetic abnormality of Cu metabolism (Gaetke *et al*., 2014). On the other hand, due to its capacity to catalyse the production of hazardous reactive oxygen species (ROS), Cu levels beyond specific limits can potentially be deleterious (Scheiber *et al*., 2013). In addition to acute Cu toxicity in humans (Franchitto *et al*., 2003), unfavorable health consequences and stunted growth have been noted in a wide spectrum of living creatures, including both plants and animals (Amuda *et al*., 2016).

Microalgae as agents for bioremediation of heavy metals

Microalgae are beneficial organisms of study as they are key indicators of the toxicity of certain pollutants (Omar, 2010). Microalgae use in bioremediation has only been studied for the past 40 years, making the practice of phycoremediation, the use of algae for environmental remediation, a fairly new and innovative bioremediation method (Renuka *et al*., 2015). These primary producers are available in abundance around the globe, they are cost-effective, grow in both fresh and saltwater conditions, and are characteristically known for having a high heavy metal retention capacity (Bulgariu and Gavrilescu, 2015). The cell walls of microalgae play a key role in heavy metal bioremediation as seen in figure 1 (Poo *et al*., 2016). The cell walls typically contain various amounts of starch, glycogen, cellulose, hemicellulose and other polysaccharides that contain specific functional groups which are involved in the binding of metal ions in the biosorption process (Bulgariu and Gavrilescu, 2015). Other factors cooperating in the biosorption process include the number of active sites on the surface of the biosorbent, the accessibility and state of the molecules, as well as the affinity for a given functional group (Bulgariu and Gavrilescu, 2015).

Both living and non-living microalgae are used in the removal of heavy metals from sites of contamination, varying in their potentials. Live microalgae, provide limitations such as their dependence on pH and levels of heavy metal contamination; whereas, nonliving algae do not and are accompanied by various practical benefits: can be stored at room temperature, highly resistant biosorptive chemical coating, unaffected by toxicity; and can control biosorption capacity by adding chemical properties. Live algae tend to accumulate larger amounts of heavy metals compared to non-living biomass; however, a non-living microalga does not need a food source or other requirements.

A variety of microalgae species find niches in the heavy metal removing environment, just as all organisms must to survive and thrive off their surroundings. Genus *Chlorella* and *Tetraselmis* are renowned for their Cd-removing efficiency (Suresh Kumar *et al.*, 2015). Other genuses such as *Planothidium*, *Spirogyra*, and *Scenedesmus* have all shown potential for heavy metal removal in different studies. However, a broad list of *Chlorella* species is reported to effectively remove a range of metals and therefore the genus *Chlorella* was selected as the focus of this review, more specifically *C. vulgaris* (Søren *et al.*, 2018).

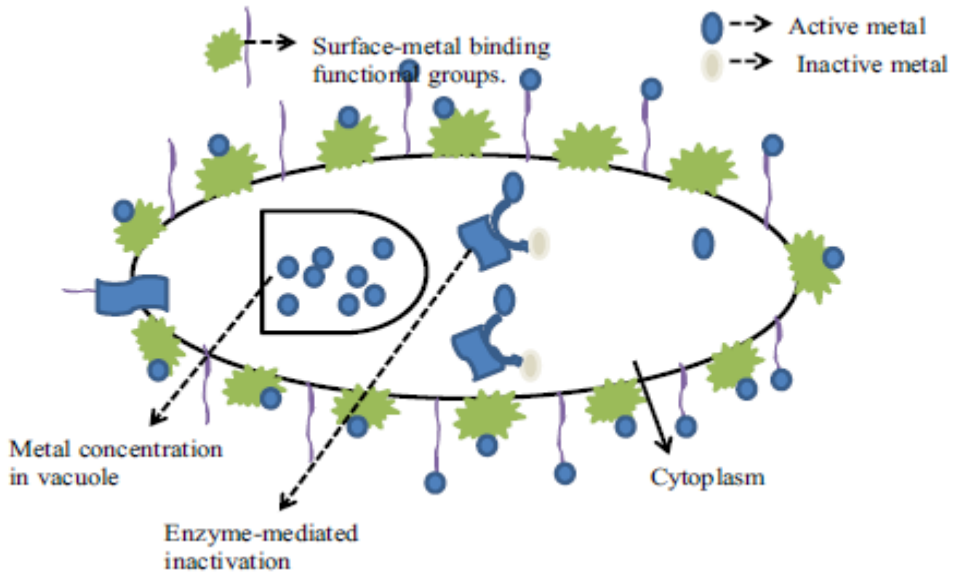
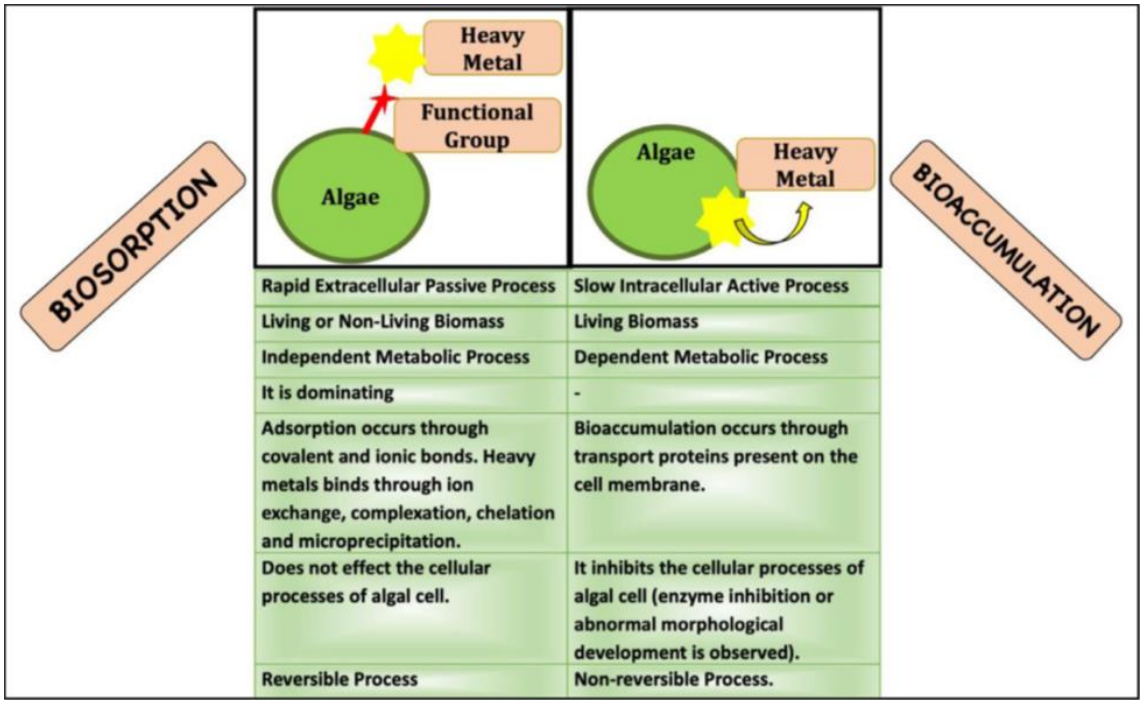


Figure.1: General mechanism of heavy metal remediation. Poo *et al.*, 2016

Phycoremediation of heavy metals by microalgae

Phycoremediation of heavy metal is a two-stage process. These techniques use bio-binding or bio-removal methods. In the first stage, heavy metals are adsorbed over the cell surface (biosorption) as shown in Fig.2. It is a rapid process and may or may not involve cell metabolism. So, this stage is known as rapid extra- cellular passive process. Algae act as potential bio-sorbents because of the presence of different functional groups on the cell surfaces. Owing to their microscopic nature, microalgal cells are able to biosorb heavy metals very efficiently as they have a high surface area and high binding affinity. Algal cells possess the capability to bind up to 10% of its total biomass with metals. Pollutants bind to the cell surface through electrostatic force of attraction and complexation (Bando *et al.*, 2019; Mohita *et al.*, 2022). This process does not require any energy, therefore; it is referred

to as a passive process. Biosorption efficiency is governed by various factors such as bioavailability of metals, availability of metal binding groups on the algal cell surface, metal uptake and storage efficiency of algal cells. Biosorption can be carried out by both living and non-living biomass. Biosorption of microalgae involve two mechanisms. It can either exchange metal ions with calcium, magnesium, sodium or potassium ions which are present on the surface of algal cells or it can form complex with the functional groups which are present on the cell surface (Yoshida *et al*., 2009). Various bio-sorbents have been studied for the effective removal of contaminants. Biosorption is more economical as it can abate pollutants with the help of non-living biomass. Contaminants can easily bind to the surface of algae due to the presence of polysaccharides, lipids, and proteins resulting in flocs formation and reducing the pollutants in the wastewater. The cell wall of microalgae consists of functional groups like sulfate, carboxyl, amino, and hydroxyl which are the binding sites for the pollutants (Hala *et al*., 2014). In the second stage, heavy metals are transported across the cell membrane to cytoplasm or other organelles. This helps in the accumulation of the heavy metals inside the algal cells and this process is known as bioaccumulation (Fig.2). Bioaccumulation is an active process and thus, requires energy for the accumulation of pollutants inside the cell (Mohita *et al*., 2022). This process involves metabolic activities for remediation thus, can be carried out by living biomass only. This process is also known as slow intracellular active accumulation because it is a slow process and requires energy for the accumulation of heavy metals inside the microalgal cells. The algal biomass thus obtained with the help of these processes, can be further used for the recovery of some value-added products (Hala *et al*., 2014). After bioaccumulation, these pollutants are detoxified, removed or form a non-toxic complex by detoxification, compartmentalization or complexation.



Rapid Extracellular Passive Process	Slow Intracellular Active Process
Living or Non-Living Biomass	Living Biomass
Independent Metabolic Process	Dependent Metabolic Process
It is dominating	-
Adsorption occurs through covalent and ionic bonds. Heavy metals binds through ion exchange, complexation, chelation and microprecipitation.	Bioaccumulation occurs through transport proteins present on the cell membrane.
Does not effect the cellular processes of algal cell.	It inhibits the cellular processes of algal cell (enzyme inhibition or abnormal morphological development is observed).
Reversible Process	Non-reversible Process.

Figure. 2: Two stage mechanism: remediation of heavy metals (Mohita *et al.*, 2022)

Phycoremediation of lead

Naturally, the bio-removal process of heavy metal ions (including Pb) in uncontrolled conditions involves two processes, namely the passive uptake and active uptake. Passive uptake better known as the bio-sorption process occurs when heavy metal ions are scattered on the cell surface, ions will bind to the surface of the cell based on the ability of its chemical affinity (Suhendrayatna, 2001; Maitina *et al.*, 2006). This process occurs when heavy metal ions bind to the cell wall in two different ways: 1) the exchange of divalent and monovalent ions such as Ca, Na, and Mg contained in the cell wall is replaced by heavy metal ions, and 2) the complex formation of ionic heavy metals with functional groups such as carbonyl, amino, thiol, hydroxyl, phosphate, and hydroxy-carboxyl which are in the cell wall (Suhendrayatna, 2001; Wardhany *et al.*, 2011; Hala *et al.*, 2014). The bio-sorption process is reversible and fast. This alternating process of bonding heavy metal ions on the cell surface can occur in dead cells and living cells. The active uptake process can occur in various types of living cells simultaneously in line with the consumption of metal ions for the growth of microorganisms or the intracellular accumulation of these metal ions (Suhendrayatna, 2001).

Phytoplankton (including *Nannochloropsis oculata*) contains O, N, and S. Organic ligands can form strong enough bonds with Pb dissolved in the aquatic environment (Pumamawati *et al*., 2013). *Nannochloropsis oculata* can be used as chelating agents for heavy metals dissolved in water bodies. Some organic compounds in the body of phytoplankton, including chlorophyll, can bind heavy metals to form complex compounds through groups that are reactive to heavy metals such as sulfhydryls and amines. Complex bonds in these compounds cause heavy metals to become very stable and accumulate in *N. oculata* cells. However, organic compounds which play the role as ligands are not the same in every type of phytoplankton, depending on physiological conditions (Imani *et al*., 2011; Rahman *et al*., 2019). Through the active process *N. oculata* can synthesize metal-chelating proteins (Purnamawati *et al*., 2013).

The absorption capacity of each microalgae cell has a difference because the surface area of each microalgal cell can affect the rate of absorption of heavy metals. *N. oculata* is a bio-sorbent that can be used to absorb the water contaminant (Yoshida *et al*., 2009; Imani *et al*., 2011). The cell wall is the most important part of the cells defense mechanism because the cell wall is the first barrier to the accumulation of toxic heavy metals. The process of accumulation of metal ions tends to settle in the cell because the value of the rate of metal release is smaller than the rate of absorption. The absorbed and accumulated toxic substances in cells will be broken down and excreted, stored or metabolized by organisms depending on the concentration and chemical potential of these materials. Hydrophilic chemicals such as Pb, Cd, Hg, Cu, and Co are usually more easily excreted than lipophilic metals. But despite this some of them can still be tightly bound to certain places of the cell, and consequently accumulate (Oram, 2014).

The absorption ability of these microalgae is due to the presence of carboxylic, sulphate, amine, and amide functional groups in cell walls that are known to be capable of binding to metal ions. Ionic bonding occurs between the negative charge in the functional group inside the microalgae cell wall and the positive charge of the heavy metal Pb ion (Wardhany *et al*., 2011). The carboxyl and amine groups play a greater role in the absorption of metal ions by *N. salina* (Hala *et al*., 2014). This group has a lone pair of electrons that can be used to interact with metal ions. Interactions that occur can be through several mechanisms such as chelation, ion exchange, reduction reactions and complex reactions (Hala *et al*., 2014; Sirakov and Velichkova, 2014). Other studies have revealed that heavy metals can be transformed through processes of complexion, absorption, methylation and changing the

value of valence even though the nature of the metal is non-biodegradable (Imani *et al.*, 2011). The same thing was also found that the ability or chemical affinity power possessed by a cell based on the distribution of metal ions around the microalgae cell causes the bonding of metal ions that are scattered around the cell wall (Pumamawati *et al.*, 2013). In a research carried out by Lud *et al.*, 2020, showed that within seven days of culture the higher the Pb level dissolved in the water, the higher the efficiency of Pb absorption by *N. oculata.*

Phycoremediation of chromium

The biological technique of heavy metal removal involves the breakdown and/or reduction of hazardous heavy metal ions to significantly less dangerous forms utilizing the living and/or dead biomass of microorganisms, (such as bacteria, fungi, algae, and yeast). Potentially useful techniques for the neutralization of Cr contamination and bioremediation of polluted water are the biological breakdown of Cr(VI) to Cr(III) **(**Cervantes *et al.*,2001). Chromium removal by microalgae is a sustainable remedy that is healthy and environmentally friendly. The biosorption of Cr(VI) using microalgae, (growing, resting, and dead cells) and biological and agricultural waste materials has been described as a possible alternative to the current conventional methods of industrial wastewater detoxification (Sen *et al.*, 2010). Chromium-resistant microalgae are responsible for the biological reduction of Cr(VI) to less mobile Cr(III); and their consequent precipitation may be an important method of detoxification of polluted Cr(VI) sites and have a possible application for bioremediation (Focardi *et al.,* 2013). Microorganisms extract heavy metals in such a way that they use the metal ions for their advancement and by changing them into carbon dioxide, methane, water, and biomass through enzyme-catalyzed metabolism of the poisonous substances (GracePavithra *et al.*, 2019). As noted earlier, hexavalent chromium is more hazardous to plants, animals, and aquatic species when compared to the trivalent species. Hence, transforming the more toxic Cr(VI) species from the solution to the less toxic one can take place by different techniques through varied conditions. In addition to extracellular adsorption and intracellular accumulation, Cr(VI) can importantly change and lower to less toxic Cr(III) species by chromium reductase enzyme as illustrated in fig.3 (Yen *et al.*,2017). More importantly, the biomass surface with electron donating agents such as hydroxyl groups, amine groups, and secondary alcohol groups interact with Cr(VI) favourably at acidic pH and is reduced to Cr(III), which subsequently binds to the

negatively charged functional groups (such as sulfonate group and carboxyl group) (Leong and Chang, 2020). Although the conversion of hexavalent chromium to trivalent chromium occurs most frequently in living biomass, it has also been seen in dead microalgae biomass, possibly as a result of glutathione being released (Yen *et al.*, 2017). In this approach, biomass made from dead microalgae was used to reduce Cr(VI) under acidic pH conditions.

Microalgae are efficient and cheap biosorbents due to their high growth rate compared to the terrestrial plants and can complete an entire growing cycle in very few days (Predawn *et al.*, 2019). Due to statistical analysis on algae potentiality in biosorption, it has been reported that algae absorb about 15.3%–84.6% compared to other microbial biosorbents (Derco and Vrana, 2018). In terms of Cr uptake, it has been shown that the capacity of *S. acuminatus var biseratus* and *S. protuberans* were very high; (96 and 90%, respectively), strongly supporting a previous study in which *Scenedesmus* sp. extracted more than 98% of Cr in a water environment (Ballen-Segura *et al.*, 2016). *Cyclotella* sp. was illustrated to have a high tendency for both Cr and Cd absorption, reaching 99-100% similar to the earlier observation of Morin *et al.*, (2007, and 2008). Also, an earlier study comparing the growth of *Scenedesmus quadricauda* under Cr(VI) stress at distinct inoculum concentrations and under autotrophic and heterotrophic conditions showed that in autotrophic cultures, the removal efficiency of Cr(VI) at 5 ppm initial concentration was 41.0%, 79.3%, 84.5% and 98.1% for 0.15, 0.3, 0.4 and 0.8 g L^{-1} inoculum concentration, respectively. In contrast, under heterotrophic conditions, at 0.15, 0.3, 0.4 and 0.8 g L-1 of inoculum, the removal efficiency of Cr(VI) was 82.4%, 95.8%, 99.6% and 99.9%, respectively (Guo *et al.*, 2020). The positive effect of high microalgae inoculum concentration on heavy metal extraction had been earlier confirmed Guo *et al.*; (2020).

Heavy metal assimilation by microalgae comprises passive biosorption by dead biomass and active biosorption by living microalgae cells. In the procedure of passive biosorption, metal ions in the cationic form are physically adsorbed over the microalgae cell surface made up of functional groups such as hydroxyl (-OH), carboxyl (-COOH), amino (-NH2), and sulfhydryl (-SH). During active biosorption, the metal ions are translocated across the cell membrane into the cytoplasm (Nouha *et al.*, 2016; Chai *et al.*, 2020). The biosorption process is most likely caused by deprotonated sulfate and carboxyl groups, as well as monomeric alcohol and laminarin, among other binding functional groups. (Bertagnoll *et al.*, 2014).

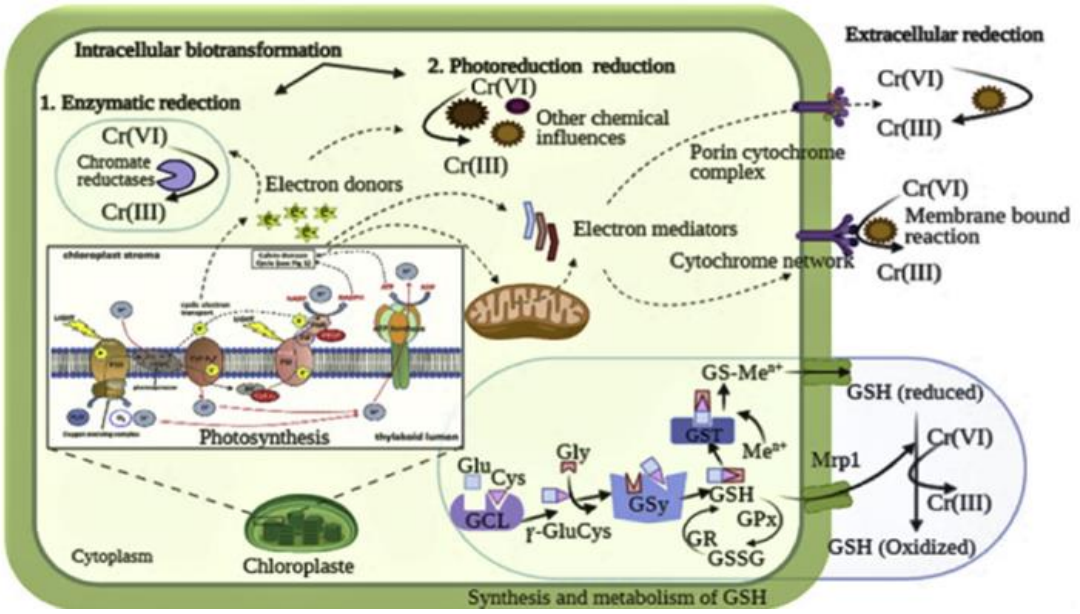


Figure.3: Mechanisms for Cr(VI) biosorption and biotransformation by microorganisms (Yen *et al.*, 2017 ; Rahman and Thomas, 2021)

Phycoremediation of mercury

Mercury is one of the most toxic heavy metals, even it's converted by- products are considered to be hazardous for the ecosystem. Microalgae are considered to be good adsorbers of mercury because of the presence of surface-active functional groups (Mohita *et al.*, 2022). Solisio and co-workers observed interaction between mercury and extracellular functional groups present over the surface of *C. vulgaris* and stated that this is the electrostatic interaction due to the presence of oxygen lone pairs. *C. vulgaris* is able to adsorbed 17.49 mg/g mercury with the help of carboxylic groups present over the surface of algae at pH 5, 20 °C within 2 h (Salisio *et al.*, 2019). Huang *et al.*; (2019) used *Scenedesmus obtusus* XJ-15 for the adsorption of mercury by the hydroxyl, carboxyl and phosphate containing functional groups. They further enhanced the removal efficiency by treating the algal biomass with phosphate and achieved 95 mg/g of sorption capacity at pH 5, 25 °C within 180 min (Haung et al., 2019). Other than extracellular removal mechanisms, enzymatic reduction of Hg2+ into zerovalent mercury with the help of mercuric reductase is also used for the abatement. Now, this Hg^0 is volatilized with the help of biological (diatoms or green algae) or non-biological (light) methods. It is observed that bio- logical volatilization is rapid (30 min to few hours) than non-biological methods. This

volatilization can be further enhanced by using chemical agents or transgenics. Bioaccumulation of mercury induces phytochelators (thiol, methyl groups) and help in the removal of mercury (Leong and Chang, 2020; Mohita *et al*., 2022).

Phycoremediation of arsenic

Detoxification of As in microalgae can be successful by several mechanisms, such as adsorption on cell surface and intracellular biotransformation, including As(III) oxidation, reduction of As(V) to As(III), complexation with thiol compounds, and sequestration into vacuoles(Wang *et al*.,2015).

The negatively charged functional groups found in microalgae cell walls; including carboxyl, hydroxyl, carbonyl, sulfhydryl, and others, enable the trapping of metallic and metallicoid cations. Adsorption is a method used by a variety of microorganisms, including fungus, bacteria, and not just microalgae. Additionally, some microalgae generate and secrete specific polymers, primarily polysaccharides, which are collectively known as extracellular polymeric substances (EPS). The majority of these polymers function as polyanions, facilitating the adsorption of metals and metalloid cations, including As as shown in fig.4 (Danouche *et al*., 2021). As(V) was discovered to be the predominant arsenic species in microalgae cells' cytosolic fractions, making up to 99% of the total As (Duncan *et al.,* 2015). It has been postulated that microalgae are able to oxidize As(III) to As(V). Utilising distinct species and strains of microalgae denote the importance and crucial impact of environmental phosphate concentration on As absorption, toxicity, and biotransformation (Wang *et al*., 2017). For example, in the halotolerant microalga *Dunaliella salina*, the accuracy of As extraction by this microalga varies under different phosphate regimes. In this regard, Christobel and Lipton (2022) evaluated the capacity of various microalgae, including the red algae *Gracilaria corticata*, for the extraction of Arsenic (As) from an aqueous solution; the results indicated that the optimum pH was 6, extraction of arsenic was 90.2%, and maximum assimilation capacity was 2.21Mmol/g. It was discovered that *Cladophora* species continue to assimilate arsenic from water till the 10th day after which desorption starts. Maximum arsenic uptake was realised to be 99.8 % (Wang *et al*., 2017). Maximum removal efficiency of this toxic element was 40.7% of As (*S. almeriensis,* pH 9.5, and 3 hours). Other results described that *C.vulgaris* was able to extract up to 70% and tolerated 200 mg/L of As^{5+} present in the growth medium (Jiang, 2011).

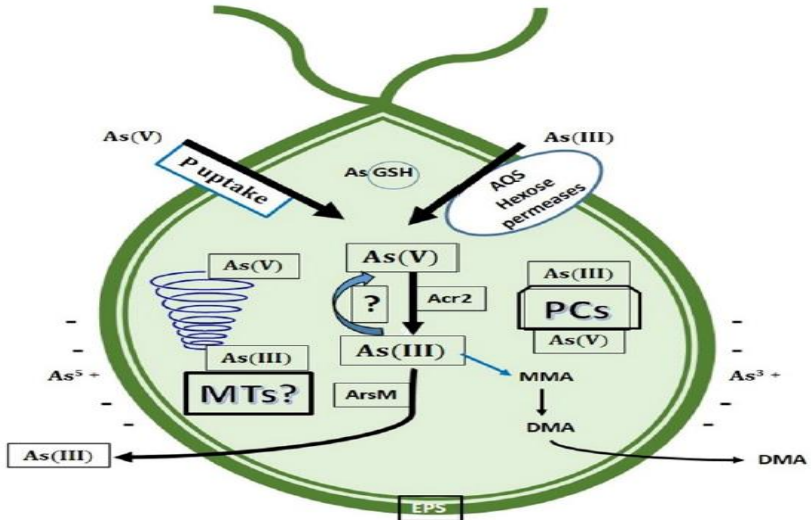


Figure. 4: Uptake and resistance mechanisms to As in microalgae Danouches *et al.*, 2021, Zhang *et al.*, 2021

Phycoremediation of cadmium

It has been seen that different strains of microalgae have different effect towards cadmium toxicity. Cadmium decreases growth as well as photosynthetic efficiency and enhances the secretion of chelators which helps in the detoxification of cadmium. Algae secrete phytochelatins or enzymes as a self- protection mechanism against toxicity of cadmium. Deng *et al.*; (2009) observed that low concentration of cadmium has minimal effect on the growth of *Microcystis aeruginosa.* However, higher concentration of cadmium causes the secretion of more malondialdehyde content and enzymes like esterase, mutase which inhibits growth as well as photosynthetic activity of the algal cell (Deng *et al.*, 2019; Mohita *et al.*, 2022). Out of two green algae (*Scenedesmus accuminatus var biseratus* and *Scenedesmus protuberans*) and diatom *Cyclotella* sp., diatoms showed maximum tolerance as well as better remediation of cadmium (99%) (Vo *et al.*, 2020). A research carried out by Chandrashekharaiah *et al.;* (2021) observed cadmium phycoremediation capacity of two freshwater algae *Scenedesmus acutus* and *Chlorella pyrenoidosa. S. acutus* showed maximum removal of 57.14% of cadmium where 98.5% is adsorbed over the surface of the cell and only 1.5% is accumulated inside the algal cell (Chandrashekharaiah *et al.,* 2021). Similarly, marine red algae *Sarcodia suidae* was able to bioadsorb more cadmium as compared to bioaccumulation. Han *et al.;* (2020) observed the ratio of biosorption/ bioaccumulation rate in the dark and light phases for 1 and 5 mg/L concentration of cadmium. They observed

maximum abatement in the light phase for both concentrations. In the light phase, biosorption/bioaccumulation ratio is 1.75 and 1.74 for 1 mg/L and 5 mg/L, respectively. They also observed that as the bioaccumulation rate increased with time, algal tolerance decreased, photosynthetic and respiratory efficiency is reduced (Han *et al.*, 2020). For the enhancement of the removal efficiency various innovative techniques are used for the modification of the biomass which leads to increase in the removal efficiency. It can be concluded that cadmium abatement capacity depends upon the strain of the bioremediators, mechanism of remediation, manipulation technique and on the removal parameters (Mohita *et al.*, 2022).

Phycoremediation of copper

Metal sequestration in microorganisms happens in two steps: the first is typically quick and independent of cell metabolism, involving the adsorption of metal ions onto cell walls as a result of interactions between positively charged metal cations and negatively charged cell wall molecules; the second, known as the rate-limiting step, is slower and only takes place in living cells, where metal ions are actively transported across the cell membrane and enter the cell via ion pores, channels, and other openings. (He *et al.*, 2014). In rare cases, the process by which metals are taken up by cells involves ions either binding to multivalent ion carriers or, after doing so, chelating proteins that allow the ions to enter the cell by endocytosis. (Narula *et al.*, 2015). To diminish heavy metals toxic impact, certain microalgae can create chemicals that interact with heavy metals outside the cell in order to decrease their bioavailability and entry in the cell; once inside the cell, some microalgae can expel again heavy metals or internalize them into vesicles and/or organelles (Quigg, 2016). For instance, it has been demonstrated that *Skeletonema costatum* may adsorb Cd+2 or Cu+2, which have been added to the culture media, in vacuoles to lessen the toxicity of the heavy metals. The *eustigamophycean Nannochloropsis oculata*, for example, was able to totally collect up to 0.25mM of Cu, but the majority of it was afterwards removed by metabolism (Martinez-Macias *et al.*, 2019). Although there are many methods used by microalgae to combat heavy metal toxicity, the creation of thiol-containing peptides is thought to be the most specific and intriguing.

Indeed, cysteine-rich polypeptides play a key role in cell defence against oxidative and metal stress. The sulphide groups present in the side chain of cysteine residues possess a negative

polarity resulting in electrochemical affinity towards heavy metal cations; in addition, sulphide is a strong reductant able to react with reactive oxygen species protecting the cell against oxidative stress. Glutathione, the simplest cysteine-rich peptide, provides protection against oxidative stress (Kwon *et al*., 2019) and has been occasionally reported to be released after exposure to heavy metals (Ahner *et al*., 2002) Phytochelatins (PCs) are polypeptides made of 2 to 10 GSH units and are enzymatically synthesized: GSH is transformed into glutamylcysteine, which is then changed into PC (Musgrave *et al*., 2013). PCs are known to attach heavy metals and to carry them within the cytosol indirection to vacuole, (Peng *et al*., 2014). The terrestrial green alga *Stichococcus* minor was shown to have high levels of GSH and glutamylcysteine when growing in the presence of 10 M Cu, pointing to the importance of PC precursors in Cu detoxification (Kalinowska and Pawlik-Skowro-ńska, 2010). Zhang *et al*.; (2015) differentiated the release in PCs by *Chlorella sp.* after subjection to Cu, Cd and Pb and established the highest PC content in cultures spiked with Cd.

However, since they can remove and hold onto a greater amount of metals utilizing both biosorption and bioaccumulation mechanisms for a longer period of time, living algae cells are more effective at treating wastewater than dead biomass. The heavy metals extraction capacity of various microalgal species in varieties of wastewater sources (e.g., municipal, petrochemical, electroplating, and dairy) For instance, *Spirulina* sp. remediated 91 and 98% of Cu^{2+} and Ca^{2+} after cultivation in municipal wastewater, (Al-Homaidan *et al.* 2015; Anastopoulos and Kyzas 2015). When grown in municipal wastewater, the extraction capacity of *Chlorella minutissima* was 62, 84, 74, and 84% for Zn^{2+}, Mn^{2+}, Cd^{2+}, and Cu^{2+}, respectively (Yang *et al*., 2015). After cultivation in oil sands tailings ponds, *Cladophora fracta* extracted 99% of Cu^{2+} and 85% of Zn^{2+} (Mahdavi *et al*., 2012). After growing in acid mine drainages, the bioremediation efficiency of *Oedogonium* sp. was 46, 34, 48, and 50% for Cu^{2+}, Ni^{2+}, Zn^{2+}, and Co^{2+}, respectively (Bakatula *et al*., 2014). In Vasant Kunj, New Delhi, India, arsenic (As) was totally remediated from drinking water by the filamentous green alga *Cladophora* (Jasrotia *et al*., 2014). *Chlorella* sp., *Scenedesmus* sp., and *Chlamydomonas* sp. Have been shown to be active in remediating heavy metals, some hazardous organic compounds, and secondary pollutants from wastewaters with a wide range of initial effluent percentage (Gao *et al*., 2016; Yang *et al*., 2016). Terry and Stone, 2002 reported that living *S. abundans* had the ability to remediate Cu up to 99% from aqueous solution.

Conclusion

Heavy metals are environmental contaminants that are non-biodegradable in nature and are hazardous to humans as well as flora and fauna. These pollutants are released directly or indirectly by several industries like electroplating, dyeing, batteries and automobile into various water bodies. Phycoremediation is the most reliable and sustainable technology as compared to conventional technologies used for the treatment of waste water. Microalgae have been shown to have the capacity to potentially abate pollution of industrial waste waters by heavy metals.

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