Review

Cyanobacterial Extracellular Polymeric Substances for Heavy Metal Removal: A Mini Review

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Abstract: Heavy metals from various natural and anthropogenic sources are becoming a chief threat to the aquatic system owing to their toxic and lethal effect. The treatment of such contaminated wastewater is one of the prime concerns in this field. For decades, a huge array of innovative biosorbents is used for heavy metal removal. Though extensive microbes and their biomolecules have been experimented and have showed great potential but most of them have failed to have the substantial breakthrough for the practical application. The present review emphasis on the potential utilization of the cyanobacteria for the heavy metal removal along with the toxic effect imposed by the pollutant. Furthermore, the effect of significant parameters, plausible mechanistic insights of the heavy metal toxicity imposed onto the cyanobacteria is also discussed in detail. The role of extrapolymeric substances and metallothionein secreted by the microbes are also elaborated. The review was evident that the cyanobacterial species have a huge potential towards the heavy metal removal from the aqueous system ranging from very low to very high concentrations.

Keywords: cyanobacteria; heavy metal; toxicity mechanism; extrapolymeric substances; metallothionein; life cycle assessment

1. Introduction

The increase in the world's population has led to the exploitation of bioresources to overcome the demand for water, land, food, and energy, resulting in cascading the effects on both environment and human health [1]. The continuous human growth and their requirement have led to the boom of industries, which resulted in the manufacture of various toxic chemical compounds. Their inapt dumping in the environment has caused contamination of soil and water [2]. The increased industrialization also resulted in an imbalance for both flora and fauna [3]. Various pollutants such as heavy metals, polycyclic aromatic hydrocarbons, halogenated aromatic compounds, benzene, toluene, ethyl benzene and xylene (BTEX) compounds, etc., are continuously being released from petrochemicals, oil refineries, pharmaceutical, agrochemical industries, etc. [4]. The accrual of these compounds in the environment has adversely affected the yield and fecundity of soil, water, and air, thus, imposing severe health issues to living biota. Owing to these compounds' persistence and non-biodegradable nature, they continue in the ecosystem for

longer duration and cause toxic effects on both soil microbial diversity and plant growth. Besides, the accumulation of these compounds in different parts of the plants has led to their transport in the food chain [5,6]. Owing to the carcinogenic and mutagenic nature of these compounds its accumulation in human bodies with time can result in serious illness. Thus, there is an imperative prerequisite to regulating the discharge of these compounds into the environment.

Regardless of the various regulatory steps being undertaken to limit the discharge of toxic pollutants into the environment, pollution levels are still hazardous. Numerous methods have been implied to remove these harmful compounds, viz. physical, chemical, and biological. Physical and chemical methods such as chemical precipitation, ion exchange, adsorption, reverse osmosis, electrodialysis, ultrafiltration, nanofiltration, coagulation, flocculation, floatation, etc., are effective, but they are not economic and release secondary pollutants, causing the obliteration of soil properties and fertility. Therefore, a cost-effective and ecofriendly method should be developed for the removal of these toxic pollutants. Biological treatment is currently in fashion for metal remediation from the environment [7,8]. Bioremediation is a unique survival method by microorganisms or plants under stress conditions that they gain the ability to break down and utilize complex toxic compounds such as metals, polyhydroxybutyrate (PHA) into a non-toxic form. The use of photosynthetic bacteria for bioremediation of contaminant is an ecofriendly and cost-effective technique [9].

Cyanobacteria can mineralize or remove toxic contaminants occurring naturally or xenobiotically. They are well-capable in adapting in the adverse environment, ranging from halophilic to cryophilic [9]. The outer layer comprised of extrapolymeric substance (a polysaccharide) possessing several binding sites for environmental remediation. It is also capable to synthesize the metal-binding proteins (metallothioneins) that are capable to bind with the inorganic pollutants such as heavy metals via cysteinyl thiolate bridges to cysteine ligands [9]. They can easily be cultivated in different types of wastewaters such as freshwater, brackish water, and industrial wastewater as a potential medium for growth. It can also be very clear from the scientific search engine data such as Figure 1 represents the annual number of publications with the phrase "cyanobacteria for Heavy Metal Removal" over the past five years (2016–2020). It shows a clear rising trend towards the potential application of cyanobacteria in the present time for the environmental remediation. Therefore, the present review discusses the heavy pollution and its toxic effect on surface water, groundwater, humans, and onto the environment. Further, aiming at a potential of cyanobacterial-derived composite polysaccharides based biomaterial (i.e., extrapolymeric substances) for heavy metal removal from waste and polluted sites.
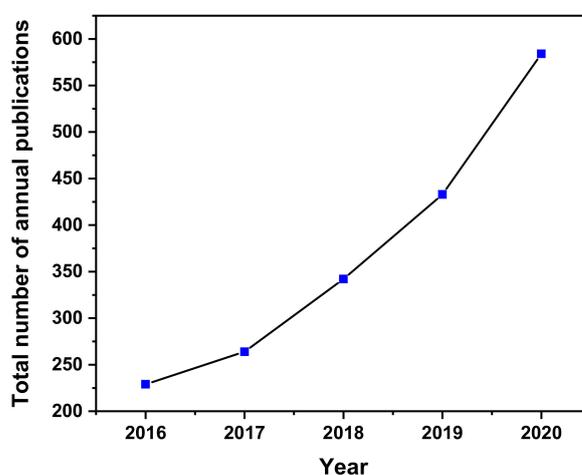

Figure 1. Annual number of publications with the phrase "cyanobacteria for Heavy Metal Removal" over the past five years (2016–2020) assessed on 15 December 2020.

2. Heavy Metals as Pollutants

Heavy metal (HM) is the term generally utilized for metals and metalloids having an atomic density of 5 g/cm^3 that includes arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), nickel (Ni), copper (Cu), iron (Fe), selenium (Se), lead (Pb), palladium (Pd), mercury (Hg), cobalt (Co), zinc (Zn), etc. [10–12]. With the rapid industrialization and large-scale mining activities, HM comprising wastewater is directly or indirectly discarded into the ecosystem leading to ecological imbalance. This further imposes deleterious health issues to all the flora and fauna that pertain to the deterioration of the environmental quality due to the high toxicity of the heavy metals [13]. They are non-degradable and can accumulate in the living organisms and their food chain. European Economic Community (EEC) have positioned Cd, Hg and their substances in most hazardously lethal substances (black list), and other HMs, Co, Cu, Pb, Zn, Ni, Cr, etc., and their substances are placed in less hazardous substances (grey list). According to EPA these HMs and their compounds have been identified as "priority contaminants" based on their toxicity. However, some HMs viz. Se, Ni, Cu, Zn, and Co in trace amounts are indispensable for living beings since they play a vital role during metabolism as they are an essential part of various enzymes and metalloproteins. HMs viz. Pb, Cd, Hg, Ag, and As are not known for any biological functions, and therefore they are non-essential and toxic for living beings [14]. Though HMs occur naturally and are released in the environment during weathering and volcanic eruption, but due to the extensive anthropogenic activities such as tanning, mining, smelting, agricultural practices, and automobiles have intensified the HM pollution than those occur naturally [15].

In the terrestrial and aquatic environments, HMs are present as ionic species or interacts with organic and inorganic ligands and forms complex or associate with suspended matter or colloids. The concentration, speciation, bioavailability, and exposure duration of HM determines the toxicity towards living biota. Furthermore, soil and water pH are the significant factors influencing mobility, bioavailability, and toxicity. Other factors affecting the HM speciation include soil type, organic matter, particle size, various amendments, organic colloids, iron oxides, and cation exchange capacity (CEC). For instance, depending upon pH Cr (VI) can be found as chromate, chromite, and dichromate forms. Cr (III) exists in the hexa-coordinate form and has a prearrangement of octahedral ligands. Hexa-aqua-chromium is present in the strongly acidic pH condition as the main Cr species in a solution of inorganic Cr (III) salts. In surface water, Cr (III) exist as both mono and oligomeric compounds. Drinking water generally has neutral pH and barely contains organic matter, which eventually limits dissolved Cr (III) because of low solubility. If the aqueous solution contains high organic matter Cr (III) compounds exist in the monomeric form. Thus, the validation of Cr (III) insoluble nature at pH 7.0 is only applicable to those solutions that do not possess the ligands that compete with water. The detrimental effects of HMs on human health are well documented. The toxic impact of HM depends on their interaction with various biomolecules and cellular and biochemical processes for living organisms [16–18]. HM excessive accrual in the ecosystem has caused severe toxic effects on biodiversity and ecosystem functions.

Further, the Earth's surface is covered with 71% water, of which the accessible drinking water is only 0.3%. According to a report by the U.N. World Water Development Agency, this issue will be even grimmer in the upcoming future, and by 2025 half of the global population would be susceptible to water scarcity. Further, the condition is prophesied to be even more deteriorating because of the upsurge in the anthropogenic actions and direct disposal of environmental contaminants into the freshwater streamlines. The increased nutrient level in water bodies causes an increase in the growth of phytoplankton. This process is called eutrophication. It will lead to the depletion of carbon availability, leading to a decrease of aquatic diversity, decreased dissolved oxygen level, inappropriate odor and taste of water, and reduced water body aesthetic value [2]. Therefore, it is essential to restrict the release of HMs in water bodies to maintain the quality of water.

Toxicity of Heavy Metals on Human Health

Humans are exposed to HMs via contaminated foods and drinking water consumption, inhalation of HM polluted air, or working in HM contaminated environment. The contamination to HMs follows a cyclic chain, i.e., from industries to the air, water, soil, plants and foods, and humans and animals. The entry of HM in the human body follows a route such as Pb, Cd, Mn, and As enters the human body via the gastrointestinal route. In contrast, other HMs enters via inhalation of contaminated air. HM causes various health toxicity to the human body. These include damage to the functionality of the brain, lungs, kidney, and liver, neurotoxicity, cancer, coma, and even lead to death. The health toxicity caused by various HMs is listed below.

The exposure to Cd leads to Cu, Zn, and Mg imbalance in the body and urine [19]. Enhanced Cd level in the body causes anosmia, renal failure, neurological disorders, problems in the male reproductive system, osteal disorders, and cancer. The exposure to Cd has similar nephrotoxic effects on both men and women but women and kids are more susceptible to cadmium exposure because of the lesser iron storage and upregulation of Fe channels in women and kids, which leads high Cd uptake since both Cd and Fe has analogous uptake mechanisms [20]. Workers working in Cd rich environment suffer from urolithiasis (kidney stones), and with the progression glomerular and renal failure occur [21]. The exposure of liver to Cd causes swelling in hepatocyte and damage to lysosomes (membrane bound) thereby induces hepatotoxicity. Further, Cd exposure also causes impairment to mitochondrial cristae, infiltration in inflammatory cells, hypertrophy of Kupffer cells [22]. Cd damages the renal Ca, PO_4 transport, metabolism of vitamin D, and parathyroid hormone. When exposed to Cd > 5 mg/m^3, it damages lung epithelial cells; long-term Cd exposure destroys nasal epithelium and olfactory function [23]. Cd causes inflammation in the lungs influencing the synthesis and release of various signaling molecules such as cytokines, adhesion molecules, and leukotrienes/prostaglandins [24]. Initial reports showed the Cd effect on the nervous system, including vertigo, headache, and sleep disturbance. At the cellular level Cd causes oxidative stress and DNA damage. Cd causes atherosclerosis, which results in cardiovascular diseases and enhanced progression [25].

The toxic effect of Cr on humans is primarily due to easy absorption of Cr (VI) in the gastrointestinal tract, lung, and certain levels over the skin. When the cell exposed directly or near to the Cr (VI), then the cell adapt mechanism for detoxification such as reduction, while reduction of Cr (VI) assist in stimulating Cr toxicity if it occurs inside or adjacent to the cells of an organ [26]. If reduction of Cr (VI) to Cr (III) extracellularly, this is not quickly transported to the cell, and hence toxicity is not observed over the cells. The amount and rate of Cr (VI), which enters into the cell and produces its toxic effect, ultimately depends upon the equilibrium between Cr (VI) extracellular and Cr (III) intracellular [27]. Cr (VI) within the cell can undergo any physiological changes and can be reduced by H_2O_2, glutathione reductase, ascorbic acid, and produces reactive intermediate Cr species like (V), (IV), finally (III), and thiyl or hydroxyl radicals. DNA, protein, and membrane lipids can be attacked by any of these Cr species, disrupting the cellular integrity and its functions [27].

Arsenic poisoning causes acute and chronic effects. Acute poisoning includes the obliteration of gastrointestinal tissues, blood vessels, and affects brain and heart. Chronic poisoning causes skin keratosis and pigmentation [28]. The exposure to the low concentration of As causes nausea, vomiting, decreased erythrocytes and leukocytes production, blood vessels damage, and abnormal heart rate. Long-term contact causes skin lesions, neurological disorder, peripheral vascular and pulmonary disease, hypertension, cardiovascular ailment, cancer, and even leads to death [29]. Pb poisoning often causes gastrointestinal tract infections and damage to the central nervous system [14]. Acute conditions include appetite loss, headache, fatigue, insomnia, abdominal pain, vertigo, renal failure, arthritis, and hypertension. Chronic conditions lead to mental disorders, congenital disabilities, psychosis, autism, weight loss, paralysis, dyslexia, brain and kidney damage, coma, and death [15].

3. Cyanobacteria: An Outlook for Environmental Remediation

Cyanobacteria are well-known microbes that are found in almost every habitat viz. aquatic, terrestrial (forests, grasslands, and deserts), and extreme environments [30]. They are unicellular and photosynthetic in nature and have been found 2.5–3.5 Ga years ago on the Earth [31]. Mostly, these are genetically diverse and are classified as the class single phylum cyanobacteria (Oxyphotobacteria) in the Gram-positive bacteria, division eubacterial (Gracilicutes), and class Cyanophyceae/Myxophyceae in the algal division Cyanophyta. Cyanobacteria are sometimes considered in algae as per the rule of ICBN (International Code of Botanical Nomenclature) but are true bacteria. They are significant organisms both ecologically and economically with prospective implementation in different field viz. aquaculture, agriculture, bioenergy and biofuels, pharmaceuticals, human nutrition, and pollution control depend on applications [32]. They have enormous adaptability to varying environmental conditions like desiccation, salinity, UV radiation, HMs, high light intensity, oxidative, extreme conditions, and form algal blooms. Figure 2 represents the network visualization of terms associated with cyanobacteria and heavy metal with at least 10 occurrences of the associated keywords. It depicts the present trends in research and development regarding the application of printed circuit board in the Web of Science.
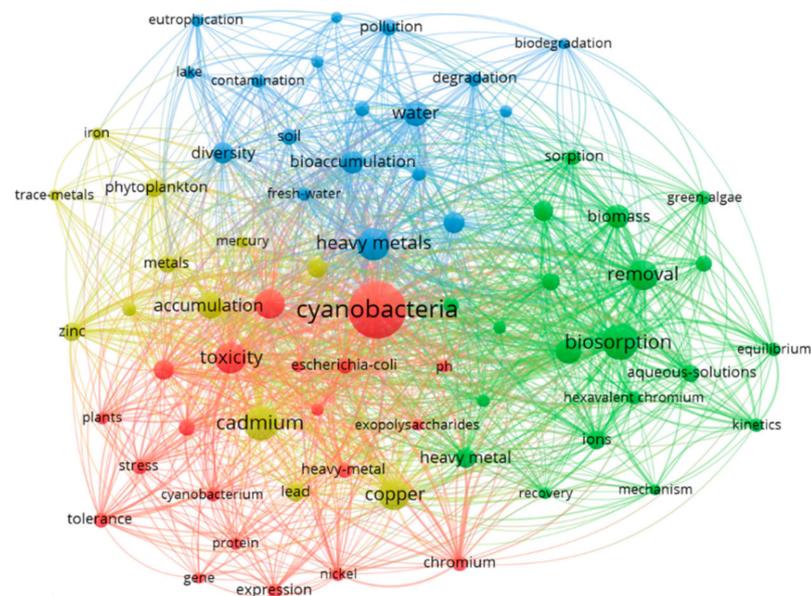
Figure 2. Network visualization of terms associated with cyanobacteria.

Cyanobacteria-mediated remediation of wastewater is economical, efficient, and ecofriendly as compared to other technologies. They are readily available in large amounts in the aquatic environment and many other habitats. They have a high affinity for metals/pollutants. Table 1 represents the literature review available on various cyanobacterial species utilized for the heavy metal removal process.

Table 1. Literature review on various cyanobacterial species utilized for the heavy metal removal process.

S. No.	Cyanobacteria	Modified Support	Adsorbate	Aqueous Phase (pH)	References
1.	*Oscillatoria*	Immobilized Ca-alginate beads	Cd	6	[33]
2.	*Nostoc minutum* and *Anabaena spiroides*	-	Pb, Cd, Ni	-	[34]
3.	*Pseudanabaena catenata*	-	Sr	10.5–11.5	[35]
4.	*Arthrospira platensis*	Immobilization alginate, silica gel, or agarose	Pb	4–5	[36]
5.	*Graesiella emersonii*	-	U, Ra	4	[37]
6.	*Cyanobacterium Metallothionein*	Immobilized with graphene oxide and SiO$_2$	Cd	8 and 6	[38,39]
7.	*Nostoc* sp.	-	Cr	6	[40]
8.	*Pithophora oedogonia* and *Spirogyra neglecta*	-	Pb, Cu	5	[41]
9.	*Scytonema*	Dimethylformamide slurry	As	6.9	[42]
10.	*Chroococcus multicoloratus* and *O. trichoides*	-	Pb	5 and 5.14	[43]
11.	*Lyngbya putealis* HH-15	Sodium alginate, calcium chloride	Cr	2	[44]
12.	*Nostoc punctiforme* A. S/S4 and *Chroococcidiopsis thermalis* S.M/S9	-	U, Cd, Ra	4, 2, and 7	[45]
13.	*Spirulina platensis*	-	Zn	-	[46]
14.	*Pseudanabaena catenate*	-	Sr	10.5, 11, or 11.5	[47]
15.	*Lyngbya wollei*	-	Cu	-	[48]
16.	*S. platensis* and *Aphanothece flocculosa*	-	Hg	6	[49]
17.	*S. muticum*	-	Hg	5	[50]
18.	*Tetraselmis chuii* and *Spirulina maxima*	-	Cd	-	[51]
19.	*Arthrospira platensis*	Immobilization in alginate, silica gel, or agarose	Pb	4–5.5	[36]
20.	*N. muscorum*	-	Cr	3	[52]
21.	*Oscillatoria* sp., *Phormidium* sp., *Lyngbya* sp., *Aulosira* sp., and *Scytonema* sp	-	Cu, Cd, Pb	5	[53]
22.	*Synechococcus* sp.	-	Cd	8	[54]
23.	*Cyanothece*	-	Cu, Cr, Ni	5	[55]

Effect of Heavy Metal on Cyanobacterial Physiological and Biochemical Processes

The usage of several chemicals has led to contamination of the environment by toxic chemicals and HMs, which has a detrimental effect on cyanobacterial growth [56–58]. Several metals such as Zn, Cu, and Fe are indispensable for the growth of cyanobacteria since they are a crucial portion of numerous enzymes that carry out an important part in metabolic processes. However, at high concentrations, they have a toxic effect on cyanobacteria's growth [59]. HMs such as Pb, Cd, Cr, As, Hg, Ni, etc., have a toxic effect

even at low concentration on the cyanobacteria. The various physiological processes, such as growth and development, pigment contents, and photosynthetic rate are affected by HMs in cyanobacteria. Reactive oxygen species (ROS) generation can have both positive and negative effects. ROS acts as a signaling molecule at low concentration and helps in development processes, whereas when present at high concentration, they have toxic effects. The effect of HMs on the cyanobacterial cell is represented in Figure 3.
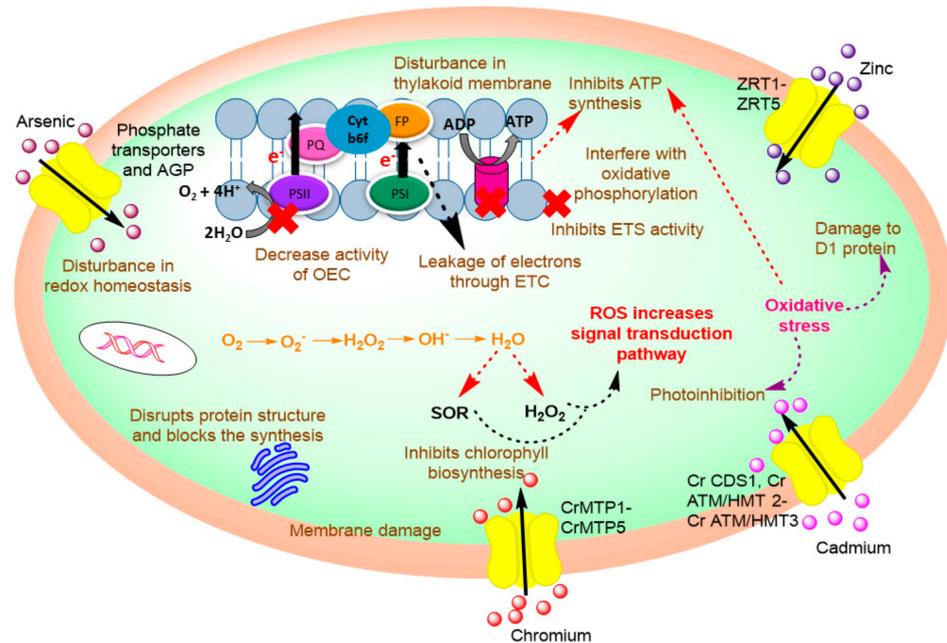

Figure 3. Sites of action of heavy metal and their toxicity in cyanobacteria.

HMs have a detrimental effect on cell growth and development. Several researchers reported a decrease in cyanobacterial biomass accumulation under HM stress [60,61]. The competition between HM and essential elements for the enzyme binding sites and transporters causes an imbalance in the cells' functionality, thereby decreasing the growth and ultimately leading to cell death [62]. However, HM toxicity depends on various factors such as HM concentration, oxidation state, and duration of exposure. Studies showed that 1 mg/L Pb concentration displayed a negative effect on *Cyanothece* and *Anabaena* growth (Heng et al. 2004), whereas 0.2 mg/L exhibited a toxic impact on *S. platensis*-S5 growth [63]. Likewise, in the presence of Cu (II) *Chroococcus* sp. growth was repressed [64]. The reduced growth can be attributed to the decrease in nitrogen metabolism, photosynthesis, etc. The photosynthetic rate is directly associated with pigment contents and, thus, biomass accumulation. HM have a detrimental effect on light-harvesting systems (carotenoids, chlorophyll (chl), and PC), which unswervingly affects the photosynthetic processes and disrupt the chl structure [65]. The decreased pigments contents are due to ROS generation, which inhibits chl biosynthesis [66] (Figure 4). The chl a content of *Nostoc* was inhibited in the presence of Cr, which reformed the photosynthetic activity [67].

HM contamination adversely affects the vital constituents of the photosynthetic system. The primary is the water-splitting complex, which is affected by HM even at low concentration. Prasad et al. (1991) reported that at high HM doses, the toxicity intercedes further oxygen-evolving complex (OEC) and photosynthetic system II (PS II) [68]. Furthermore, Perreault et al. (2009) observed that HM also disturbs the proteins synthesis involved in light harvesting systems, PS I, and PS II, and downregulates the expression of genes involved in carbon fixation [69]. The biochemical photosynthesis originates from light absorption by antenna pigments and transported to the reaction centre (RC) for photochemical reactions. The energy transfers from one PS to another leads to ATP and NADPH generation, which is a source of energy during CO_2 assimilation. The surplus

energy absorbed by pigments is released as heat identified as fluorescence [70]. HM toxicity is assessed via fluorescence transient test, which delivers evidence about HM effect on energy flux and electron transport. HMs (Hg, Pb, As, Cr, etc.) affect the electron transport chain, so energy dissipation occurs.
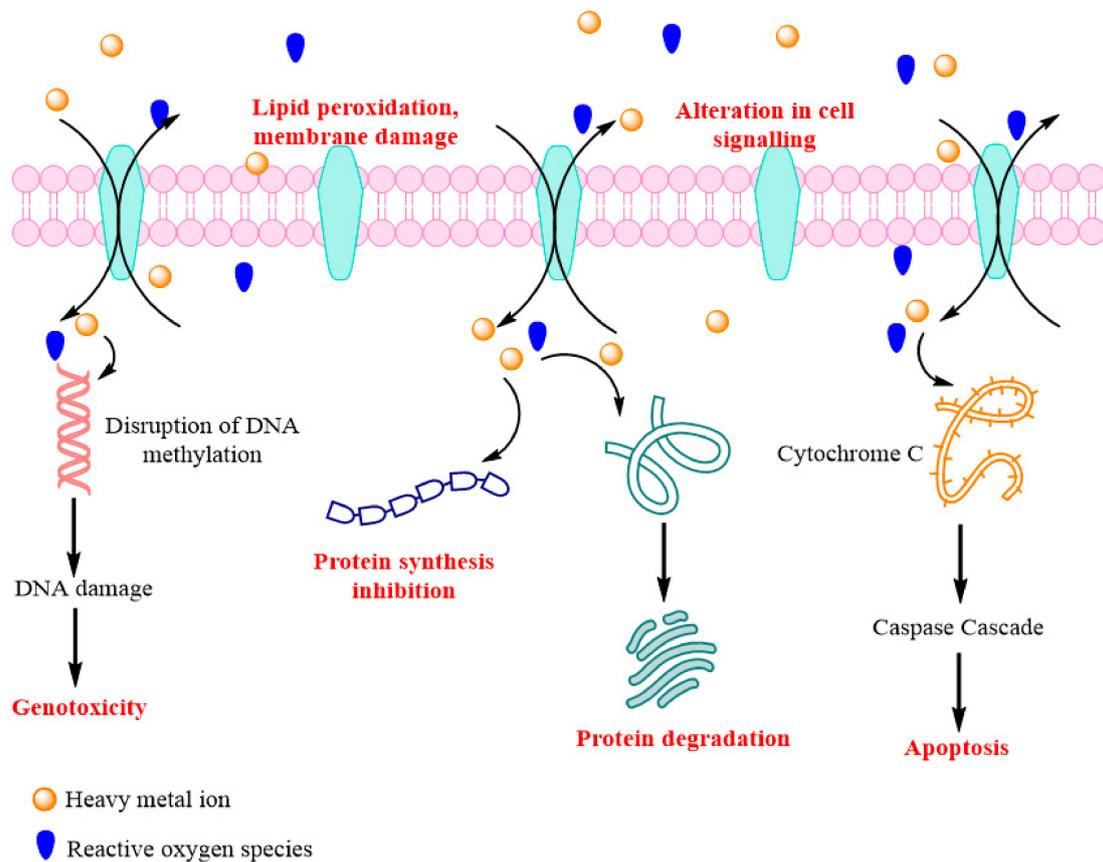

Figure 4. Mechanism of heavy metal toxicity at the cellular level.

The active RCs of *Microcystis aeruginosa* showed a decrease in number when treated with As (III) (1 mg/L) and to sustain the excess energy equilibrium between absorption and utilization, which leads to enhanced energy flux parameters [71]. Studies suggest that HMs have two major target sites, i.e., PS I and PS II [72]. The inhibition of D1 protein under HM stress decreases the activity of PS II [73]. Moreover, HM also affects phosphorylation processes (cyclic or noncyclic). Cox and Saito (2013) revealed that proteins associated with photosynthesis are affected under HM stress; some are downregulated, such as ferredoxin-NADP oxidoreductase, PsbU, and putative thylakoid lumen peptidyl-prolyl cis-trans isomerase [74]. In *Chlamydomonas*, the respiration rate was decreased when treated with 4 mM As (III) while 0.30 mM As (III), no decline was observed [75]. In the presence of As and Cr enhance respiration activity was observed in *Chlamydomonas*, which can be attributed to the energy management process and over a generation of ROS [67].

4. Heavy Metal Removal by Cyanobacteria

The removal of HMs from the environment by cyanobacteria is a novel and cost-effective approach. Cyanobacteria take up these pollutants (discharged from various industries into the environment) to enhance the metabolism and decrease/remove the contaminant from the environment. Cyanobacteria can be used as wild-type, mutant, or genetically modified forms for bioremediation of HMs [76]. The presence of diverse proteins and polysaccharides on the cyanobacterial surface helps bind with the HMs with enhanced efficiency [77]. Biosorption has various advantageous, including cost-effective,

the selective expulsion of species, recovery plausibility, higher recuperation probability, no sludge production, desorption ease, and high adsorption rates. Cyanobacteria are the utmost diverse group of photosynthetic prokaryotes and can be present in marine, freshwater, and terrestrial. Cyanobacteria outer layer is composed of polysaccharide, i.e., extracellular polymeric substances (EPS) having an enormous number of binding sites for the adsorption of organic and inorganic pollutants.

Cyanobacterial EPS: Structure and Occurrence and Role of Metallothionein

Cyanobacteria can synthesize the metal-binding proteins, which is known as the metallothioneins (MTs). Small molecular weight proteins are rich in cysteinyl residue, which binds to the metal ion through cysteinyl thiolate bridges to cysteine ligands. MTs are usually found to be associated with Zn and Cu; however, they also bind with other toxic metals such as Cd, Hg, and Pb. Figure 5 represents the domain nature of metallothionein. The metal binding property of MTs is mainly due to cysteine residues and their characteristic arrangement viz., -Cys–Cys–Cys–X–Cys–or –Cys–X–X–Cys (X: amino acids). Studies show the elevation in synthesis of MTs in high HMs concentration [78].
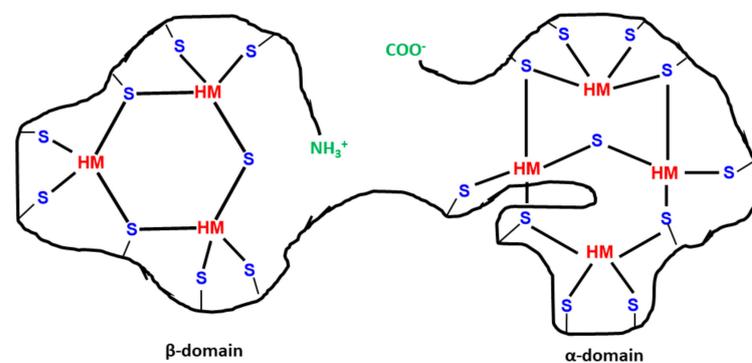

Figure 5. Domain nature of metallothionein (adapted from [78]).

The synthesis of MT-like proteins has been described in *Anacytis nidulans,* which was correlated with resistance towards Cd (II). In other cyanobacteria such as *Synechocystis* sp. PCC 6803 and *Anabaena* sp. PCC 7120 ZN-MT-like sequence was present [79]. In *Synechococcus* sp. PCC 7942 shows the upregulation of metal-binding MT and heat-shock stress protein in the presence of Cd (II), Cu (II), and Zn (II) (1 µM). Cd showed a superior effect among all metals, and authors stated that to overcome the acute metal stress, heat-shock proteins were upregulated while the continued exposure upregulated the MTs [80]. El-Enany and Issa (2000) reported *Nostoc linckia* was tolerant for Cd and Zn due to the sequestration of these metals by metal-binding proteins [81]. Likewise, a metal-binding protein in *Anabaena doliolum* provides resistance against metal and other environmental stresses [82].

The high affinity of Apo-MT for Zn is due to the presence of sulfhydryl groups. Since sulfhydryl groups are reactive, Zn is transferred to another protein from MT due to the exchange reaction. Thus, the sulfhydryl affinity towards Zn makes MT an effective scavenger for metal ions. MTs have a regulatory role in the stimulation or deactivation of several molecular effectors. For example, Apo-MT scavenges Zn from transcription factor IIIA, thus deactivates TFIIIA.

The molecular weight of cyanobacterial EPS ranges from 10 kDa to 2 MDa with a complex structure due to branched monomers and regularly with different compounds [83]. Ostensibly, the cyanobacterial EPS portion is made of a minimal of six different kinds of building blockers. It is intriguing that the EPS composition delivered from microalgae or bacteria is chemically contrasting in relation to EPS of cyanobacteria. Hexoses, galactose, pentose, ribose, glucose, mannose, xylose, arabinose, acidic hexoses, deoxy hexose,

rhamnose, methyl rhamnose, galacturonic corrosive, and so forth are the primary structure blockers typically seen in cyanobacterial EPS [84].

Moreover, Rossi and De Philippis (2016) reported various sorts of building blockers, for example, *N*-acetyl glucosamine, 2, 3-*O*-methyl rhamnose, 4-*O*-methyl rhamnose, 3-*O*-methyl glucose, and 3-*O*-methyl rhamnose [84]. Notwithstanding, glucose is often present in cyanobacterial EPS at an elevated amount. However, there are a few reports where different sugars, for example, fructose, arabinose, xylose, or galactose, were found to be present at a higher amount than glucose [85]. Rossi and De Philippis (2015) reported uronic acid as sole building blockers in EPS from *Microcystis wesenbergii* [83]. Thus, the presence of repeatable unit of building blockers in cyanobacterial EPS is a remarkable element for specific species. These unique units are framed through 5–8 monosaccharides, which is the reason for the complex behavior of cyanobacterial EPS.

There are few studies that reported the presence of non-sugar chemical structures in EPS like hexosamines, pyruvates, acetic acid derivations, uronic corrosive, and sulphate esters. In spite of the fact that the amount of non-sugar parts is extremely low in cyanobacterial EPS, nonetheless, they give significant qualities to the EPS because of their extraordinary association to sugar moieties. The EPS negative charge is due to the presence of pyruvates, uronic acid, and acyl bunches. The presence of such non sugar moieties in EPS bestows binding and adsorptive properties [86]. Gel like consistency of EPS is because of sulfates. There are a few metal particles like Ca^{2+} and Mg^{2+} that were present in EPS of cyanobacteria, which differentiate them from both bacterial and diatom sources. The divalent cations are cross-linkages between the EPS sugar particles, which is responsible for higher constancy and stability [87]. EPS from cyanobacteria are hetero-polymers, which are made of lipids, proteins, monosaccharides, and DNA. Generally, they are called lipopolysaccharides or glycoproteins. Owing to the presence of a different amount of uronic acids, proteins, pyruvic corrosive, and O-methyl, O-acetyl, and sulphate groups and their linkage in different situation in side sugar moieties, cyanobacterial EPS are a bit intricate to understand [87].

5. Strategy for Enhancing the EPS Production

Worldwide, the EPS production from cyanobacteria has gained substantial attention and has been applied in the biotechnology field. Cyanobacteria are cultivated in a controlled environment, basically in artificial cultivation units. Generally, bioreactors are constructed as closed and open. Moreover, depending on the usage, they can be divided as laboratory-scale and commercial. However, various factors such as land, nutrients, water or energy cost, cyanobacterial strains, methods undertaken for harvesting, climatic conditions, light source, and other axenic conditions affect the EPS production [88]. The EPS production from cyanobacteria can be carried out either using the wild or recombinant strain. Figure 6 represents the stepwise process strategy for enhancing the EPS production.

5.1. Wild Strain

A lot of studies have been undertaken for the production of EPS using cyanobacteria of wild type for their application in industries. However, proper isolation and identification of cyanobacteria are required for the desired product. Additionally, the microbe should have a suitable property for the synthesis of the desired product. During the EPS coproduction, the formation of toxic products is not desirable. The main challenges faced during pilot-scale production of EPS is to isolate the potential cyanobacterial strain, which is quite time consuming and tedious. The main advantage of the wild microbial strain is its robustness during industrial production conditions. However, the major demerit of using the wild strain is the low production of EPS. The nutritional requirement for each microbe is different; thus, organic compound consumption by microbial diversity under natural conditions should be adequately assessed. The isolation, screening, and characterization of novel strain is a highly tedious process. The identification of new strains is carried out via 16S rRNA gene sequencing. Various cyanobacteria strains have been isolated for production

of high yield EPS, such as *Synechocystis*, *Microcystis*, *Chamaesiphon*, *Cynotheca*, *Chroococcus*, *Cyanobium*, *Gloeocapsa*, *Gloeobacter*, *Gloeotheca*, and *Synechococcus*. Several bacterial strains are also described as promising candidates for EPS production. Still, cyanobacteria are a potential candidate for the production of EPS commercially since it can yield EPS from CO_2, light, and water. Additionally, they have a high tolerance towards a vast range of light intensity, temperature, and desiccation [89].
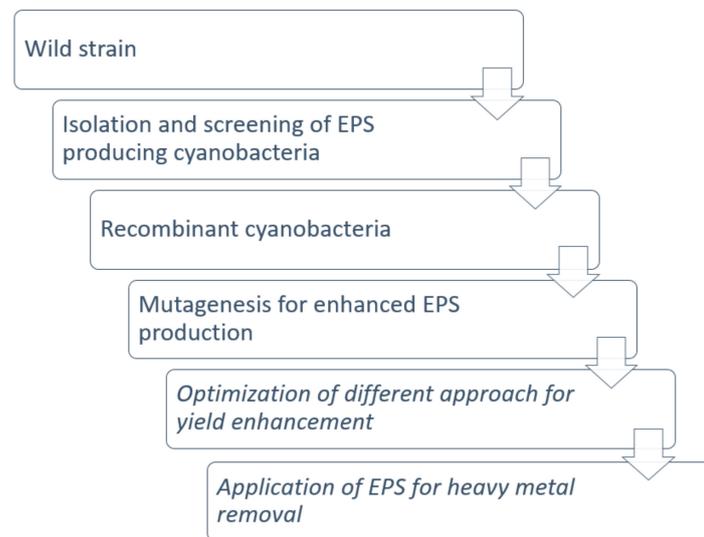
Figure 6. Stepwise process strategy for enhancing the extracellular polymeric substances (EPS) production.

5.2. Isolation and Screening of EPS Producing Cyanobacteria

Numerous factors affect the EPS yield from cyanobacteria. Generally, from different places, mat samples are collected. The thickness of the mat considered is 5–10 cm [90]. The samples are subcultured under controlled salinity. The light–dark cycles are maintained depending on the researcher's interest. After culturing, EPS is extracted, grounded, and compared based on the material dry weight.

5.3. Recombinant Cyanobacteria

The recombinant DNA (rDNA) technology is used to enhance the yield of EPS from engineered cyanobacteria. This technology comprises the identification, isolation, and insertion of a specific gene into a vector for the rDNA molecule construction. Further, DNA sequencing is used to confirm the microbial strain carrying recombinant plasmid [91]. The development of a recombinant microbial strain is a tedious process as the production of EPS in cyanobacteria is governed by numerous genes. Thus, extensive research is essential for the development of genetically engineered microorganisms for enhanced production of EPS. Several methods such as cloning, site-specific mutagenesis, random mutagenesis, and both have been employed to develop recombinant microbes, though iterative saturation mutagenesis is a directed evolution strategy for high EPS yield [91].

5.4. Mutagenesis for Enhanced EPS Production

The approaches such as random mutagenesis, site-specific, and site-saturation mutagenesis have been employed for engineering microbial strains for high EPS yield. Combinatorial beneficial mutagenesis (CBM™) and look-through mutagenesis (LTM™) have been used to study mutagenesis [91]. At a particular gene position in LTM, rapid screening of amino acids was done, leading to enhanced EPS production with selective properties. In CBM, individual mutations were identified. Studies proved that CBM™ and LTM™ are more advantageous than the conventional mutagenesis study [91].

5.5. Optimization of a Different Approach for Yield Enhancement

Generally, the biorefinery approach can be applied to reduce the cost of EPS production by cyanobacteria. The EPS production can be enhanced by metabolic engineering or improving the photobioreactor design, growth conditions, and harvesting method. The cyanobacterial growth depends on several factors such as temperature, nutrient content, pH, water, light source, gaseous exchange, and ion concentrations. These factors can vary depending on the cyanobacterial strain used for EPS production. Various strains survive at extreme conditions, i.e., the temperature below zero or temperature >70 °C [92]. Indeed, mostly cyanobacteria are mesophilic organisms but require different growth conditions when grown in the laboratory.

Yoshimura et al. [93] investigated NaCl's effect (200 mM) on EPS production in *Nostoc* sp. HK-01 and *Anabaena* sp. PCC 7120. It was observed that *Nostoc* sp. released 65% of the dry weight capsular EPS while *Anabaena* sp. released 18% of the dry weight. They also observed that in the presence of NaCl, the ratios of sugars composition in Anabaena's EPS barely changed when compared to control (without stress condition). However, in *Nostoc* sp., the composition of the sugar of EPS changed significantly under NaCl stress. The intensity of the light also affects the cyanobacterial growth, thereby influences the EPS production. As respiration and photosynthesis are significantly dependent on light intensity; thus, the EPS production is directly correlated with them [94]. Ge et al. (2014) observed that the light intensity significantly influenced the total EPS from *Microcoleus vaginatus*, and with the increase in light intensity EPS production was enhanced [95].

5.6. Application of EPS for Heavy Metal Removal

Microbes remove HMs from wastewater via the bioaccumulation or adsorption process. In the adsorption process, the charged macromolecules present in the cell envelope binds with HMs. Since EPS carries a negative charge; thus, cyanobacterial strains producing EPS can be considered as a potential candidate for removing positively charged HMs. In cyanobacteria, the peptidoglycan layer is much thicker and has high polysaccharidic crosslinking compared to Gram-negative bacteria. Thus, this inimitable property of cyanobacteria cell walls makes them a promising candidate for HM removal. Biosorption is a complex physicochemical process, while adsorption includes ion exchange, complexation, adsorption, precipitation, etc. Diengdoh et al. (2017) isolated *Nostoc muscorum* and studied its potential for Zn^{2+} removal from aqueous medium [96]. They observed that removal of Zn^{2+} followed the first-order adsorption isotherm, and the binding between EPS and Zn^{2+} was proved by energy-dispersive X-ray (EDX) study. FTIR study revealed the presence of various negatively charged functional groups such as sulfhydryl, hydroxyl, carboxyl, carbonyl, amine, and alcohol on the EPS surface. Mota et al. (2016) investigated the removal of Pb^{2+}, Cd^{2+}, and Cu^{2+} by *Cyanothece* sp. CCY0110. Results showed that *Cyanothece* sp. has high removal efficiency for HMs [97].

The natural capability of cyanobacteria to produce a high yield of EPS has made them a promising candidate. Presently, the maximum percentage of commercially available EPS is from cyanobacteria. EPS derived from cyanobacteria is comprised of six or more different forms of building blocks. The cyanobacterial EPS molecular weight ranged from 10 kDa to 2 MDa. Cyanobacterial EPS contains a high quantity of the acyl group, uronic acid, and pyruvic acid, which helps bind and adsorb HMs. EPS's high stability is mainly due to the crosslinking of sugar molecules that are imparted by divalent cations. The high conductivity of EPS is due to the presence of charged and polar amino acids. Since extensive research has been focused on the isolation of potential cyanobacterial strain for the production of EPS, further study is desirable to better understand the chemical structure of EPS for better application in various sectors.

6. Different Factors Assessment for the Wastewater Cultivated Cyanobacteria

In recent times, research and development at the interface of functional composite materials for renewable and sustainable technologies have resulted in ecofriendly, green,

and sustainable materials to continue the transition from fossil fuel-based economy to waste-derived sustainable circular economy and industries [98,99]. This resulted in reduced carbon footprint arising from the waste-derived green carbonaceous materials [98–102].

6.1. Life-Cycle Assessment and Risk Assessment Analyses for Industrial Application

Various literature has utilized the life-cycle assessment (LCA) and risk assessment analyses for illustrating the environmental and energy performance of wastewater-based cyanobacteria [103]. Bussa et al. [104] compared the LCA evaluation on the cyanobacteria with maize as the potential feedstock for the polylactic acid production. Cyanobacteria outperformed maize in several aspects viz. land utilization, non-toxic to marine, terrestrial and freshwater, etc. Recently, the cyanobacterial cultivated onto wastewater was compared for their biofuel application via comparing the lipid extraction, anaerobic digestion, and combustion techniques. It is also being recommended to work on the strategies for reducing the energy demand, carbon capturing, and media nutrients. Further, well-to-pump LCA, i.e., in general, a defining terminology utilized for the transportation fuel LCA, the results reported by Sander and Murthy [105] depicted that the total energy inputs for cyanobacterial and algal-derived biodiesel is lower in comparison to the petroleum diesel. The biofuels and biomaterials derived after the utilization of biomass (cyanobacterial/microalgae, etc.) cultivated using wastewater could be an inherent ecofriendly option for greenhouse gases (GHG) emissions [106]. Here, each step includes smart coproduct use, decarbonization of the electricity, and heat grids and indirect energy requirements for fertilizer, transport and building material, etc., that when taken into consideration will fully reduce/mitigate the heat cost and GHG emissions [107,108].

6.2. Limitations of Wastewater-Cultivated Cyanobacteria

Cyanobacteria are capable of forming thick mats with the available wastewater containing even little quantity of nutrients causing "the blooms" [109]. Owing to the additional nitrogen and phosphorus generated via the human activities, cyanobacterial blooms have accelerated over the decades with the globalization and industrialization. This thick bloom mat blocks out the light and oxygen needed by aquatic organisms further cursing their survival to the native environment. In addition, the summer and warm conditions results in the bloom die resulting in a foul smell of the water bodies [110,111]. Further, the cyanobacterial species may act as a carrier for transferring the radionuclides, heavy metals, pesticides, herbicides, toxic, carcinogenic, and mutagenic agents [110,112] both in the marine and atmospheric environment. The pollutants might be lethal to the microbes as well, further restricting its utilization in any kind of ingestion even to the cattle, and aquatic organisms.

7. Conclusions and Future Perspectives

Owing to the different merits and edges, bioremediation has risen as an appealing option in contrast to the current traditional remediation techniques that has enormous application in ecological administration and contamination control.

➢ In spite of the fact that the methods of poisonous metal biosorption and microbial biosorbents have been broadly examined in labs, the improvement of reasonable biosorption advances and their usage in different divisions delivering substantial metals have not increased as desired. Nonetheless, a predetermined number of organizations have created and popularized microorganism based bioremediation advances.

➢ Assessment and improvement of biosorbent materials from microbial biomass is a quickly expanding thirst area in academia and in industries.

➢ Cyanobacteria are a captivating and extraordinary class of microorganism with wonderful versatility, omnipresence, and assorted variety. In spite of the fact that the presence of cyanobacteria in the terrestrial and in the aquatic environment are easily available, screening and determination of the promising cyanobacterial species/strains (isolation and selection) with a high metal sorption limit is a demanding task.

The encouraging species/strains can be an appropriate possibility for the improvement of new and productive biosorbents, which can be packed in the biosorption column so as to completely use the capability of cyanobacteria in bioremediation of toxic metals, endeavors ought to be coordinated towards screening and determination of promising species/strains, advancement of more effective biosorption segments with better execution, minimal effort immobilization and mass culture procedures, and hereditary control of cyanobacteria for the upgrade of metal biosorption or uptake capacity and overexpression of genes encoding metal-binding and surface proteins. Henceforth, the waste-derived biomass for environmental remediation application resulted in advancement of the ecofriendly, green, and sustainable materials further contributing to the sustainable circular economy and industries.

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