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Case Study

Bioremediation of Cadmium, Chromium and Copper Using Green Microalgae

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ABSTRACT

The past decades have witnessed rapid boom in technological advancements posing a serious concern over the safety of the environment. The incessant and careless discharge of wastewater from various industries and urbanization has steadily degraded the natural resources. The heavy metals can persist and bioaccumulate in the environment. Thus, the removal of heavy metals from the environment is of prime importance. The physical and chemical methods of wastewater treatment are less preferred because of high capital cost and generation of secondary waste. The wastewater treatment using algae is known as phycoremediation. The most promising attribute of the microalgae-based treatment is the production of algal biomass. The algal biomass has myriad of applications in pharmaceutical, nutraceutical and fuel industries. Thus, the development of zero carbon footprint fuel will lessen the demand on the very scarce stock of fossil fuel, thereby promoting ecological and sustainable balance of the environment. The ability to sequester metal ions makes green algae a promising candidate for wastewater treatment. This review aims to report the potential green microalgae used in remediation of the heavy metals mainly cadmium, chromium and copper. Furthermore, strategies to enhance the heavy metal removal efficiency have also been discussed.

Key words: Heavy metal, Cadmium, Chromium, Copper, Green microalgae

Water is essential for the sustenance of life on Earth. The discharge of untreated effluents from the leather, mining and agro based industries into the waterbodies poses a potential damage to the ecosystem and human wellbeing. The heavy metals commonly discharged into the aquatic bodies are mainly Pb, Cd, As, Cr, Cu, Ni and Zn [1]. These heavy metals are lethal even at trace amounts [2]. By the virtue of their accumulating potential and persistent nature, their disposal demands a sustainable and ecofriendly approach. Phycoremediation involves the complete set of processes that transform the harmful, toxic compounds into less harmful substances by using algae [3]. Though macroalgae are used in remediation their sedentary nature, slower growth rate and offshore cultivation makes it less preferable [4]. On the other hand, Microalgae has several advantages such as simple structure, quick multiplication, rapid uptake capacity and efficient removal of heavy metal. Additionally low capital cost, avoidance of chemical reagent and abundance of biomass makes the microalgae a more green sustainable and economic choice [5].

The ability to sequester metal ions by green microalgae makes them promising tool for phycoremediation. The works of Oswald and Gotaas, Palmer are pioneers of this green technology [6-7]. Since then, a plethora of studies has focused on the usage of microalgae to remove heavy metal contaminants. Generally, the three mechanisms adopted by green microalgae to remove heavy metals are bio-uptake, biosorption and bioaccumulation. Bio-uptake occurs via micronutrient transporters and subsequently storing heavy metals in particular cellular compartments. Biosorption is biphasic; involving the adsorption of heavy metals by extracellular cell associated materials, cell wall components e.g., carboxyl, amino hydroxyl, pyruvate, phosphate groups as well as sulphate and subsequent accumulation inside the cells [8]. Numerous research investigations have demonstrated various green microalgae capable of removing heavy metals from contaminated areas.

To evoke tolerance in response to heavy metal stress, multiple signaling pathways intercommunicate up to the molecular level. The stress caused by the heavy metal activates ROS signaling, and diverse enzymes such as catalase (CAT), glutathione reductase (GR), or ascorbate peroxidase (APX), phytohormones, defense proteins such as phytochelatins (PCs), metallothioneins (MTs) and glutathione -S-transferases (GSTs), followed by phytosequestration and compartmentalization, all of which contributes to the

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remediation [9]. Focus should be directed on the development of target-based strains having the ability to accumulate higher metal concentration with minimum nutrient requirement, high biomass production and develop systematic study in the omics-based approach to engineer the target pathways and apply them to industries [4]. This review investigates the studies on different chlorophycean microalgae for removal of heavy metals cadmium, chromium and copper.

Characteristics of cadmium, chromium and copper

The studies conducted on characteristics of the heavy metals such as the oxidation forms, minimum permissible limit in drinking water, sources, sources of entry in humans and health effects are summarized in the (Table 1).

Table 1 Characteristics of the heavy metals

Heavy metal	Metal forms	Minimum limit in drinking water (mg/L)	Sources	Source of entry in human	Health effects	References
Cadmium	Cd (II)	0.005	Weathering, mining, electrochemical plating, Ni–Cd batteries, Volcanic eruption, paints, pigment, chemical fertilizers, semiconductors, smelting, alloy manufacturing, etc.	Cigarette smoking, through rice cereals, Inhalation of industrial fumes containing Cd, etc.	Irritability, reduction in bone density, pulmonary disease, abdominal pain, irritability, headache, cardiovascular problems, renal tubular osteomalacia etc.	[4]
Chromium	Cr (II) - Cr (VI)	0.1	Metal processing, tannery facilities, chromate production, stainless steel welding, and ferrochrome and chrome pigment production.	Occupational exposure	Nose ulcers, ulcers in the stomach and small intestine, anemia etc.	[10]
Copper	Cu (II)	1.3	Non-sparking tools, Drinking water, Birth control, Cookware	Presence of excess copper in drinking water or ingestion of acidic foods cooked in uncoated copper cookware	Stomach pain, nausea, vomiting, diarrhea, headache, dizziness, fatigue, fever, extreme thirst, tachycardia	[11]

Role of microalgae in metal detoxification

Phycoremediation is the green technology which uses algae for the biotransformation of toxic compounds into less toxic substances. The polyphosphate bodies in algal cells have the ability to sequester heavy metals and then further detoxify it [12]. Another detoxifying mechanism found in algae is the production of metallothioneins and phytochelatins to chelate heavy metals, resulting in inactivation of metal ion via compartmentalization in cellular compartments. The presence of sulphide groups in cysteine residues is important for vitality of the function of these polypeptides. The metal binding peptides are responsible for transporting toxic metals into various organelles and can act as free radical scavengers relieving the radicals formed during the stress. The heavy metals sequestered inside the vacuoles are chelated by organic acids such as malic or citric acids [13]. Meticulous research has prompted the discovery of many hyperaccumulator algal strains potent in phycoremediation.

Cadmium, chromium, copper removal by green microalgae

Phycoremediation, an ecofriendly sustainable tool to recover contaminated wastewater can be a very efficient and cost-effective solution for combating heavy metal toxicity. Various research investigations have shown that green microalgae can successfully remove cadmium, chromium and copper from contaminated waters, which plays a significant role in the phycoremediation process (Table II). Recently a study used *Chlorella sorokiniana* as a biosorbent to remove chromium from the industrial effluent. The results showed that microalgae removed 99.6793% of chromium (VI) upto 1000 ppm after three days [14]. Another study used metal oxide Fe_2O_3 with combined advantage as immobilizing material and adsorbent. During the study the potential of Fe_2O_3 with microalgae to remove Cr (VI), Pb (II), Cd (II), Cu (II) was evaluated and found that microalgae combined with Fe_2O_3 has higher percentage of removal efficiency compared to individual cells [15].

The efficiency of *Scenedesmus obliquus* CNW-N as bioadsorbent to remove cadmium from the aqueous solution was examined. A complete remediation of cadmium was observed at pH 6 and 30°C. Further a maximum adsorbent capacity of 68.6 mg/g, making it an efficient bioadsorbent for the removal of contaminants [16]. Many investigations were conducted on *Chlorella vulgaris* for its efficiency in removal of chromium ion from wastewater. As a result, *C. vulgaris* showed a removal of 99.75% (60 mg/50 ml of 1000 ppm) at pH 3 and contact time of 60 minutes. The data obtained from the study strongly correlated with different isotherm models such as Langmuir, Freundlich, Temkin, Redlich-Peterson, etc. It is very useful for designing technology for the remediation of heavy metal ions from wastewaters [17].

A study investigated the removal of copper using *C. vulgaris* and the results indicated that 81.97% and 92.53% of Cu were removed by living algae subsequently after 10 minutes and 12-hour exposure respectively. The remediation of municipal and hyper saline effluent is of growing concern these days [18]. Another study investigated potential of *Tetraselmis chuii* and *Pavlova lutheri* to remove heavy metals from municipal leachate and hypersaline effluent. The result showed that the microalgae are capable of removing 95% of heavy metals from wastewater [19]. A study to determine the remediation efficiency of copper by two freshwater green microalgae mainly *C. pyrenoidosa* and *S. obliquus* was conducted. According to the findings, the former removed 79.3-90.9% and latter removed 75.9-91.4% of Cu respectively after 8 days of culturing [20].

Table 2 Removal efficiency of Cadmium (Cd), Chromium (Cr) and Copper (Cu) by various green microalgae

Green microalgae	Source of algae	Heavy metal	Experimental condition	Duration	Removal efficiency (%)	References
Parachlorella kessleri Bh-2	Ilek transboundary river flowing through the Aktobe region of Kazakhstan and the Orenburg region of Russia	Cd, Cr	L2 - minimal medium 24-h period 50 μmolm^{-2} s^{-1} pH: 6.8 20 ±2°C	8 days	Cd: 89% Cr: 96.15%	[2]
Desmodesmus sp.	Muncipal wastewater treatment plant, Rome	Cu	BG-11 medium 55 ± 1.6μmol m^{-2}s^{-1} pH below 3 18±2°C	2 days	90%	[3]
Desmodesmus sp. CHX1	Local oxidation pond	Cu	Synthetic piggery digestate Batch experiment BG-11 medium pH: 5.97 30°C	4 days	88.35%	[5]
Chlorella sorokiniana	Institute of Plant Biochemistry and Photosynthesis, Seville, Spain	Cd, Cu	Tris-acetate-phospate media with acetate and ammonium concentrations (optimized) 150μEm^{-2} s^{-1} pH: 6.5-7 27°C	3 days	65%	[9]
Dunaliella salina	Sebkha of Sidi El Hani (governorate of Sousse, Tunisia)	Cd, Cr	f/2 medium 80 μmolm^{-2} s^{-1} 22 ± 2°C	14 days	Cd: 10.03-19.45% Cr: 92.38-95.63%	[21]
Chlorella thermophila (MN855377)	Sukinda mining area, Jajpur district of Odisha	Cr	BG-11 medium 18:6 h light: dark period 42 μmolm^{-2} s^{-1} pH: 3.0 27 ± 2°C	2 days	92.765%	[22]
Chlorella vulgaris (13-1) and *Coelastrella* sp. (3-4)	Water bodies in Sweden	Cd	BG-11 50 μmolm^{-2} s^{-1} pH: 7.2 20°C	1 day	*C.vulgaris*: 72% *Coelastrella* sp.: 82%	[23]
Chlamydomonas sp.	Industry runoff	Cr	Bold's Basal medium pH: 4 25°C	30 minutes	91.3%	[24]
Chlorella pyrenoidosa	National Collection of Industrial Microorganism (NCIM), Maharashtra, India	Cd, Cr, Cu	BG-11 medium 12:12 h light: dark period pH :7.5 25 ± 2°C	15 days	Cd:87% Cr: 73% Cu: 60%	[25]
Chlorella pyrenoidosa (NCIM2738) and *Scenedesmus acutus* (NCIM5584)	National collection of Industrial Microorganisms (NCIM), Pune, India.	Cd	Fog's medium Batch experiment 12:12 h light: dark period 250 μmolm^{-2} s^{-1} pH: 7.0 ± 0.5 25±1°C	8 days	*C.pyrenoidosa*: 97% *S. acutus*: 98.5%	[26]
Scenedesmus sp.	Swine wastewater	Cu	BG-11 medium 100±10 μmolm^{-2} s^{-1} pH: 7-7.5 20 ± 2°C	8 days	64.60 %	[27]
Chlorella vulgaris	Punjab Bioenergy Institute, University of Agriculture, Faisalabad	Cd, Cr, Cu	BG-11 medium 100–120 μmolm^{-2} s^{-1} 14:10 h light: dark period pH: 7.29 25±1°C	5 days	Cd: 59% Cr: 58% Cu: 45%	[28]
Neochloris aquatica RDS02	Freshwater lake at Kuppanur, Tamil Nadu and India.	Cd, Cr, Cu	Tannery effluent Bold's Basal Medium 110 μmolm^{-2} s^{-1} 12:12h light: dark period 23±1°C	15days	Cd: 94.5% Cr: 98.5% Cu: 97.9%	[29]
Desmodesmus communis and *Monoraphidium pusillum*	Algal Culture Collection of the Department of Hydrobiology, University of Debrecen (ACCDH-UD).	Cu	Jaworski's medium 40 μmolm^{-2} s^{-1} pH: 7–7.5 24°C	7 days	*D. communis*: 89% *M. pusillum*: 81%	[30]
Chlorella vulgaris and *Scenedesmus spinosus*	FICOLAB laboratory at the University of Concepción, Chile	Cu	Mine tailings water BG-11 medium 60 μmolm^{-2} s^{-1} pH: 7.29 20 ± 3°C	4 days	64.7%	[31]

Dunaliella salina	Sambhar Salt Lake, Rajasthan, India	Cr	Bold's Basal medium 10 Wm^{-2} 24h day/night light period pH: 7.5 ± 1 25 ± 2°C	5 days	66.4%	[32]
Chlorella coloniales	Paddy fields of Marvdasht in the south of Iran	Cd, Cr	BG-11 medium 60 μmol m^{-2} s^{-1} 24±2°C	Cd: 4days 15 h Cr: 4 days 12h	Cd: 97.3% Cr: 98%	[33]
Scenedesmus quadricauda	Culture Collection of Algae and Protozoa (CCAP), Scotland, UK	Cr	Bold Basal medium 110 μmol m^{-2} s^{-1} 16: 8h light: dark period pH: 2 5–35°C	4h	100%	[34]
Desmodesmus sp. MAS1 and *Heterochlorella* sp. MAS3	Non-acidophilic environment	Cd	modified Bold's Basal medium with low phosphate 60μmol $m^{-2}s^{-1}$ pH: 3.5 23±1°C	7 days	>58%	[35]
Chlamydomonas reinhardtii 11-32b, *Chlorella vulgaris* and *Scenedesmus almeriensis*	SAG Culture Collection of algae,University of Antofagasta, Chile and University of Almeria, Spain	Cu	Bristol medium enriched with a trace metal solution from F/2 of Guillard medium 12:12 h photoperiod 1000μmol $m^{-2}s^{-1}$ pH: 6.5 ± 0.1 23 ± 2°C	7 days	88%	[36]
Chlorella sp.	Local habitat	Cr, Cu	Bold's Basal medium 4000 lx 12:12 h light: dark period 27.5°C	12 days	Cr: 73.1% Cu: 45.7%	[37]
Chlorella vulgaris and *Desmodesmus* sp.	*Desmodesmus* sp.: municipal wastewater treatment plant, Italy *Chlorella vulgaris*: Culture Collection of Algae and Protozoa (CCAP, Scotland)	Cd, Cr, Cu	BG-11 medium 12:12 h light: dark period 29μmol $m^{-2}s^{-1}$ pH: 7.5 18 ± 2°C	12 days	Cd: 87% Cr: 73% Cu: 60%	[38]

CONCLUSION

The unique nature of the cell walls enhances the potentiality of green microalgae to remove the heavy metals in the wastewater. Numerous studies support the ability of green microalgae to significantly reduce and remove toxic heavy metals such as Cadmium, Chromium and Copper. The significant differences in the condition provided in the laboratory and those found in field is one of the main challenges to this technology. Thus, molecular studies on the native diversity of green microalgae found in variety of wastewaters and their tolerance levels need to be tested. Also, identification and development of consortia using potential strains should be encouraged for efficient treatment. Further research is needed to determine the specific position of transporters in the microalgae. The molecular information about the role of phytohormones, MAP kinase cascade to chelate ROS helps to develop promising target strains capable of better accumulation. Thus, critical studies using multi-omics approach is helpful in giving a crystal-clear idea of the molecular events concerning with heavy metal remediation and associated physiological responses in microalgae.

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