


Innovative Biosorption Techniques for Heavy Metal Removal Using Microalgae: A Comprehensive Review

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Abstract

Water and soil pollution represents a fundamental human issue globally. Heavy metals are one of the basic pollutants of water and soil, which can be caused and intensified by anthropogenic activities, including mining, transportation, and various industries. Due to the toxic effects of these metals on the environment, organisms, and human health, the removal or recovery of these elements from polluted environments is of particular importance. Different methods and techniques have been applied to remove these pollutants, among which, bioremediation has received considerable attention due to its eco-friendly and cost-effectiveness. Bioremediation uses the ability of various organisms to decrease or remove pollutants. Algae are among the organisms that show significant capabilities in removing different types of contaminants, especially heavy metal ions. Phycoremediation is an application of algae as bio-remediate agents, and depends on factors such as light, temperature, pH, type of pollutant, and type of taxon. Various strains are known for their ability to remediate heavy metals. The most basic methods in removing pollutants using algae are biosorption into the cell (absorption) and surface biosorption (adsorption), which uses the living or non-living mass of algae. New techniques, such as using transgenic microalgae, are among the effective detoxifying and rapidly growing methods. Genetic engineering for algae gene editing and gene silencing benefits various technologies and tools such as reporter genes, Cre-lox recombination, and CRISPR-Cas systems, modular cloning toolkits, regulatory elements, promoters, vectors, restriction enzymes, and post-transcriptional gene silencing technologies. Other novel techniques whose future on an industrial scale seems promising are the combined use of microalgae and bacteria, biochar addition, and biogenic nanomaterials generated from algae. These innovative methods offer sustainable and cost-effective solutions for environmental pollution, therefore boosting

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public health. Studies on the development and implementation of these techniques are ongoing in the world. In this paper, the ability of 78 algae species to remove 18 heavy metals has been reviewed.

Keywords: Absorption, Bioremediation, Heavy metals, Phycoremediation, Transgenic microalgae

Introduction

Environmental pollution is considered one of the most critical issues around the world. Due to the rapid expansion of urbanization, industrialization, manufacturing, and production of hazardous by-products, this problem is getting worse by the day and ultimately endangers environmental sustainability and human health (Rahman et al., 2021). Heavy metals (HMs) are regarded as one of the most considerable pollutants in soil and aquatic ecosystems (Ahmad et al., 2021). The natural weathering of minerals, as well as recent industrial and anthropogenic activities, led to the discharge of significant levels of HMs into the environment (Malik and Kaur Sandhu, 2023). Mining, smelting, and refining processes produce enormous amounts of contaminants and HMs, which could be distributed through the air and negatively affect the nearby areas (Izydorczyk et al., 2021). Furthermore, industrial emissions and automotive industries, fossil fuels, sewage sludge, household activities, and excessive use of pesticides and insecticides significantly contribute to polluting the environment with HMs (Briffa et al., 2020) (Fig. 1). Contamination of the terrestrial and aquatic ecosystems with HMs poses a significant hazard to the environment and therefore human health as a result of direct toxic impacts on living organisms

and further potential for increased exposure along the food chain (Huang et al., 2018). Numerous severe health conditions, including cancer, lung adenomas, kidney failure, neurological disorders, inhibition of enzyme activity, and infertility, are among the ailments caused by HM exposure (Alengebawye et al., 2021; Żukowska and Biziuk, 2008).

The rising content of HMs, their persistence in the environment, and potentially deleterious effects on ecological and human health demand effective remediation technologies. There are several methods for removing HMs from contaminated environments, including water and soil. Traditional methods such as ion exchange, chemical precipitation, coagulation, conventional and advanced oxidation, ultrafiltration, and electrochemical removal have some limitations like usability for limited metal ions, consuming higher energy and chemicals, and producing a considerable amount of sludge/solid waste (Razzak et al., 2022). Therefore, developing more effective and environmentally friendly solutions is very important. Today, the emergence of affordable methods in which no cutting-edge technology is required have attracted substantial attention as these methods are economically feasible for developed and developing countries. Among the new technologies used to reduce HMs is bioremediation, which has received greater attention from various communities

because of its low-cost, simple technology, and availability.

Bioremediation is a process that applies organisms' potential to clean up environmental contamination, such as wastewater, ground or surface waters, sediments, and soils (Boopathy, 2000). The bioremediation technique uses bacteria, fungi, plants, and algae to break down, remove, change, immobilize, or detoxify different chemicals and physical pollutants from the ecosystem (Bala et al., 2022). When biological agents interact with pollutants, bioremediation occurs spontaneously without the aid of any chemical catalysts. To facilitate and speed up the bioremediation process, it is vital to

generate the optimum environmental conditions (Verma and Jaiswal, 2016). The characteristics of the contaminated site have a great impact on the bioremediation process. The bioremediation process is influenced by various factors, including soil texture, permeability, pH, water-holding capacity, temperature, nutrients, and oxygen content (Boopathy, 2000).

Phycoremediation, the application of algae to remove contaminants from the environment, is recognized as an effective and affordable bioremediation technique. Microalgae are recognized as effective bioremediation agents in soil due to their rapid growth, large surface area, strong affinity



Fig. 1. Main sources of soil and water pollutants, including pesticides and insecticides, refining and smelting industries, automotive industries, mining, fossil fuels, sewage sludge, and household activities. Pictures used in this figure are adopted from (Di Capua, 2013; Khan, 2022), (<https://commons.wikimedia.org/>), (<https://www.brainhealth.scot/>), (<https://iglobalpunjab.com/>), and (<https://truthout.org/>).

for metal binding, high tolerance for various contaminants, and eco-friendly nature (Chugh et al., 2022; Yeheyo et al., 2024). They utilize natural metabolic processes through techniques such as bioconcentration and volatilization to detoxify and remediate polluted soils effectively. Their effectiveness lies in their ability to accumulate and degrade contaminants within their cellular structures. Furthermore, microalgae release exudates that support the growth of beneficial microorganisms in the soil, enhancing soil health and resilience (Yeheyo et al., 2024). Phycoremediation of water systems is primarily recognized for its ability to purify water contaminated with HMs and/or other pollutants; however, it can be integrated into a broader bioremediation strategy that benefits both aquatic and terrestrial environments. Since the biomass produced by algae following the phycoremediation is used as feedstock to generate biofuel and other valuable products, algal-based bioremediation is strongly favored (Razaviarani et al., 2022) such as wastewater treatment and bioenergy industries. Microalgae are mixotrophic microorganisms that have potential to utilize nitrogen and phosphate (nutrients). Therefore, this review encompasses the current research, advancements, and modern approaches in the phycoremediation of heavy-metal-polluted environments.

Phycoremediation of HMs

Algae are considered a new biological step that is a permanent, environmentally friendly, and affordable procedure for environmental protection (Touliabah et al., 2022). The merits of bioremediation based on algae are better production of biomass and high-

er aggregation ability, detoxification, and degrading xenobiotics and contaminants. Moreover, the produced biomass during bioremediation is cost-efficient in the field of clean energy.

Different species of algae, including macro- and micro-algae, diatoms, and cyanobacteria, can remove pollutants from soil and water. As an example, the ability of *Neochloris aquatica* in removing HMs including chromium (Cr) (88.7%), lead (Pb) (75.9%), nickel (Ni) (87.6%), cadmium (Cd) (60.4%), cobalt (Co) (52.9%), zinc (Zn) (84.9%), and copper (Cu) (54.4%) is considerable (Tamil Selvan et al., 2020). The study conducted by Ajayan et al. (2015) revealed the important effect of *Scenedesmus* sp. on reducing the HM pollution of Zn (65–98%), Pb (75–98%), Cu (73.2–98%), Cr (81.2–96%), and nutrients such as phosphate (>95%) and nitrate (>44.3%). Marine macroalgae such as *Caulerpa lentillifera* can also be used as inexpensive adsorbents to remove Cd, Cu, Zn, and Pb from aqueous solutions (Apiratikul and Pavasant, 2006). Table 1 lists 78 microalgae strains with the ability to bioremediate 18 HMs.

Diatoms, another group of microalgae, can bioremediate diverse forms of effluents due to their cellular structure and adaptive techniques. It may absorb and use different micro- and macro-elements (Marella et al., 2020). *Cylindrotheca closterium* be able to remove phthalate acid esters (PAEs) from surface sediments (Gao and Chi, 2015). Moreover, the herbicide mesotrione (an aromatic ketone) can be absorbed by *Halamphora* (*Amphora*) *coffeiformis* (Valiente Moro et al., 2012). *Nitzschia* sp., another

diatom genus, causes fast decomposition of organic matter by enhancing aerobic bacterial activity (Yamamoto et al., 2008). Besides, *Nitzschia* sp. and *Skeletonema costatum* can degrade the highly toxic polyaromatic hydrocarbons (PAHs) from sediments (Hong et al., 2008).

Some reports on the biodegradation of pesticides by algae can be found in the literature (Megharaj et al., 2000, 1994, 1987). El-Bestawy et al. (2007) illustrated the ability of the strains *Synechococcus*, *Oscillatoria*, *Nostoc*, *Cyanothece*, and *Nodularia* in degrading of the pesticide Lindane (chlorinated aliphatic pesticide). Kuritz and Wolk (1995) also reported the ability of *Nostoc ellipsosporum* and *Anabaena* sp. to degrade Lindane. Moreover, soil isolates of *Chlorella vulgaris*, *Synechococcus elongatus*, *Tetrademus obliquus* (*Scenedesmus bijugatus*), *Leptolyngbya tenuis* (*Phormidium tenue*), *Leptolyngbya* (*Phormidium*) *foveolarum*, *Kamptomonema animale* (*Oscillatoria animalis*), *Desmonostoc* (*Nostoc*) *muscorum*, and *Nostoc linckia* can detoxify and break down the organophosphate insecticides (Megharaj et al., 1994, 1987).

Acid mine drainage bioremediation using algae

Acid Mine Drainage (AMD) is considered an important source of HM pollution around the world that endangers species of plants, animals, and human life (Samal et al., 2020). Different strains of algae, especially microalgae, are used as a cost-efficient way of removing HMs these days. Some genera and species, such as *Spirulina*, *Scenedesmus*, *Chlorella*, *Cladophora*, *Anabaena*, *Oscillatoria*, *Stigeoclonium*, *Phaeodactylum tri-*

cornutum, non-living *Caulerpa lentillifera*, *Ulothrix zonata*, and *Turbinaria ornate*, are among the hyper-accumulator and hyper-adsorbent microalgae from AMD. They also produce a lot of alkalinities, which is important during HM precipitation treatment (Apiratikul and Pavasant, 2006; Bwapwa et al., 2017; Kandasamy et al., 2021). The lifeless biomass of *Spirulina* sp. can absorb Zn (86–98%), iron (Fe) (100%), Cu (38–76%), and Pb (40–78%) and decrease the acidity of AMD by enhancing the pH, as AMD has the acidic nature (Bwapwa et al., 2017). *Stigeoclonium* spp. are freshwater algae that can thrive in mine water containing high levels of HMs, particularly Zn, and are recognized for their effectiveness in removing Zn from the environment (Pawlik-Skowrońska, 2001).

The bioremediation mechanism of algae

Algae from various species can be used to break down organic contaminants. HM removal from the environment can also be accomplished through bioremediation. It is worth mentioning that the terms bioremediation and biodegradation are increasingly interchangeable (Singh, 2019). However, biodegradation is considered a natural process in nature, while bioremediation is commonly controlled to optimize the conditions for microorganisms. This process can take a few to several months to finish and is carried out in situ or ex-situ. In-situ bioremediation includes the remediation of pollutants at the site, while ex-situ involves the removal of the pollutants in another site (Gavrilescu, 2010). Ex-situ bioremediation can be employed if the environmental conditions are unfavorable for the growth of microorgan-

Table 1. List of micro- and macroalgae used for phycoremediation of HMs

Number	Algae species	Family	Metal	References
1	<i>Halamphora (Amphora) subtropica</i> (Wachnicka & E.E.Gaiser) Rimet & R.Jahn	Amphipleuraceae	Ni	(Dahmen-Ben Moussa et al., 2018)
2	<i>Dolichospermum affine (Anabaena affinis)</i> (Lemmermann) Wacklin, L.Hoffmann & Komárek	Aphanizomenonaceae	As	(Huang et al., 2014)
3	<i>Anabaena cylindrica (subcylindrica)</i> Lemmermann	Aphanizomenonaceae	Cu, Co, Pb, Mn	(El-Sheekh et al., 2005)
4	<i>Trichormus (Anabaena) variabilis</i> (Kützinger ex Bornet & Flahault) Komárek & Anagnostidis	Aphanizomenonaceae	Pb, Zn, Ca, Mn, Cr	(Abd El-Hameed et al., 2018; Ahammed et al., 2023)
5	<i>Ascophyllum nodosum</i> (Linnaeus) Le Jolis	Fucaceae	Cu, Cd, Zn, Ni, Pb	(Leusch et al., 1995; Medeiros et al., 2017)
6	<i>Chlamydomonas reinhardtii</i> P.A.Dangeard	Chlamydomonadaceae	Pb, Cd, Cu, Hg	(Bayramoğlu et al., 2006; Flouty and Estephane, 2012; Li et al., 2021; Tüzün et al., 2005)
7	<i>Chlorella sorokiniana</i> Shihira & R.W.Krauss	Chlorellaceae	Cu, Ni, Cd, Pb, Cr	(Akhtar, 2004; Akhtar et al., 2008; Husien et al., 2019; Liang et al., 2017; Petrovič and Simonič, 2016)
8	<i>Mychonastes homosphaera (Chlorella minutissima)</i>	Mychonastaceae	As, Cd, Cu, Zn, Mn, Cr	(Arora et al., 2017; Singh et al., 2012; Yang et al., 2015)

(Skuja) Kalina & Puncochárová				
9	<i>Auxenochlorella (Chlorella) pyrenoidosa</i> (H.Chick) Molinari & Calvo-Pérez	Chlorellaceae	Mn, Cu, Zn, Pb, Cd, Cr, Ni	(Kothari et al., 2022; P.S et al., 2021; Purushanahalli Shivagangaiah et al., 2021; Zhou et al., 2012)
10	<i>Chlorella miniata</i> (Kützinger) Oltmanns	Chlorellaceae	Ni, Cr	(Han et al., 2014, 2007; Wong et al., 2000)
11	<i>Chlorella</i> sp. Beyerinck [Beijerinck]	Chlorellaceae	Cr	(Shukla et al., 2012)
12	<i>Chlorella vulgaris</i> Beijerinck	Chlorellaceae	Ca, Mn, Pb, Cu, Cd, Ni, Zn, Cr, Fe	(Ahammed et al., 2023; Atoku et al., 2021; Manzoor et al., 2020)
13	<i>Pleurastrum (Chlorococcum) aquaticum</i> (P.A.Archibald) Sciuto, M.A.Wolf, Mistri & Moro	Pleurastraceae	Pb	(Liyanage et al., 2020)
14	<i>Chlorococcum infusionum</i> (Schrank) Meneghini	Chlorococcaceae	Fe, Mn	(Gomes et al., 2021)
15	<i>Chlorococcum</i> sp. Meneghini	Chlorococcaceae	Cu, Cd, As	(Qiu et al., 2006; Upadhyay et al., 2022)
16	<i>Cladophora vagabunda (fascicularis)</i> (Linnaeus) C.Hoek	Cladophoraceae	Cu, Pb, Cd	(Deng et al., 2008, 2007b, 2007a, 2006)
17	<i>Cladophora fracta</i> (O.F.Müller ex Vahl) Kützinger	Cladophoraceae	Cu, Cd, Zn, Hg	(Ji et al., 2012)

18	<i>Cladophora glomerata</i> (Linnaeus) Kützing	Cladophoraceae	Cr, Zn, Cu, Pb, Cd, Co, Ni, Fe, Mn	(Çelekli and Bulut, 2020; Khan et al., 2023; Vymazal, 1990, 1984)
19	<i>Cladophora parriaudii</i> C.Hoek	Cladophoraceae	Cu, Al, Pb, Mn	(Ross et al., 2021)
20	<i>Cladophora rivularis</i> (Linnaeus) Kuntze	Cladophoraceae	Pb	(Jafari and Senobari, 2012)
21	<i>Cladophora</i> sp. Kützing	Cladophoraceae	Pb, Cu, Cr, Cd, Zn, Ni, As	(Abioye et al., 2020; Sargin et al., 2016)
22	<i>Closterium lunula</i> Ehrenberg & Hemprich ex Ralfs	Closteriaceae	Cu	(Yan and Pan, 2002)
23	<i>Dunaliella salina</i> (bardawil) (Dunal) Teodoresco	Dunaliellaceae	Al, Cd, Cu, Cr, Fe, Zn, Mn, Ni, Pb	(Akbarzadeh and Shariati, 2014)
24	<i>Dunaliella</i> sp. Teodoresco	Dunaliellaceae	Zn, Ni	(Dahmen-Ben Moussa et al., 2018; Elleuch et al., 2021)
25	<i>Ecklonia radiata</i> (C.Agardh) J.Agardh	Lessoniaceae	Zn, Pb	(Matheickal and Yu, 1996; Zhang et al., 2022)
26	<i>Euglena gracilis</i> G.A.Klebs	Euglenaceae	Cu, Ni, As	(Tahira et al., 2019; Winters et al., 2017)
27	<i>Fucus distichus</i> ssp. Evanescens (C.Agardh) H.T.Powell	Fucaceae	Cu, Cd, Pb, Zn	(Medeiros et al., 2017)
28	<i>Fucus vesiculosus</i> Linnaeus	Fucaceae	Cu, Cd, Ni, Pb, Hg, Zn	(Brinza et al., 2020, 2009; El-Naggar et al., 2021; Henriques et al., 2017; Mata et al., 2008; V.R. et al., 2019)

29	<i>Gloeocapsa</i> sp. Kützing	Aliterellaceae	Pb	(Raungsomboon et al., 2008)
30	<i>Hydrodictyon reticulatum</i> (Linnaeus) Bory	Hydrodictyaceae	U	(Zhang and Luo, 2022)
31	<i>Laminaria digitata</i> (Hudson) J.V.Lamouroux	Laminariaceae	Cd, Cu	(Anacleto et al., 2017; Papageorgiou et al., 2008)
32	<i>Saccharina</i> (<i>Laminaria</i>) <i>japonica</i> (J.E.Areschoug) C.E.Lane, C.Mayes, Druehl & G.W.Saunders	Laminariaceae	Cd, Pb, Zn, Fe	(Ghimire et al., 2008; Luo et al., 2006; Xiao et al., 2012; Yin et al., 2001)
33	<i>Limnorphis</i> (<i>Lyngbya</i>) <i>hieronymusii</i> (Lemmermann) J.Komárek, E.Zapomelová, J.Smarda, J.Kopecký, E.Rejmánková, J.Woodhouse, B.A.Neilan & Komárková	Microcoleaceae	Cd, Pb, Hg	(Inthorn et al., 2002)
34	<i>Phormidium</i> (<i>Lyngbya</i>) <i>taylorii</i> (Drouet & Strickland) Anagnostidis	Oscillatoriaceae	Cd, Pb, Ni, Zn	(Klimmek et al., 2001)
35	<i>Micrasterias denticulata</i> Brébisson ex Ralfs	Desmidiaceae	Cr	(Volland et al., 2012)
36	<i>Microcystis aeruginosa</i> (Kützing) Kützing	Microcystaceae	Pb, Cu, Zn, Cd, Ni, Hg, As	(Chen et al., 2005; Deng et al., 2020; Huang et al., 2014; Pradhan and Rai, 2001; Rzymiski et al., 2014; Zeng et al., 2022)
37	<i>Nostoc commune</i> Vaucher ex Bornet & Flahault	Nostocaceae	Cu, Cd, Fe, Ni, Pb, Zn	(Atoku et al., 2021; Morsy et al., 2011)

38	<i>Desmonostoc (Nostoc) muscorum</i> (Bornet & Flahault) Hrouzek & S.Ventura	Nostocaceae	Pb, Cu, Co, Mn, Zn, Cd	(Abd El-Hameed et al., 2018; Dixit and Singh, 2014; El-Hameed et al., 2021; El-Sheekh et al., 2005; Hazarika et al., 2015; Roy et al., 2015)
39	<i>Nostoc</i> sp. Vaucher ex Bornet & Flahault	Nostocaceae	Pb, Cu, Cd, Zn, Ni	(Ahad et al., 2021; Rakic et al., 2023)
40	<i>Oedogonium hatei</i> N.D.Kamat	Oedogoniaceae	Cr, Ni	(Gupta et al., 2010; Gupta and Rastogi, 2009)
41	<i>Oedogonium rivulare</i> A.Braun ex Hirn	Oedogoniaceae	Cu, Cr, Pb, Cd, Co, Ni, Zn, Fe, Mn	(Vymazal, 1984)
42	<i>Oedogonium</i> sp. Link ex Hirn	Oedogoniaceae	Cr, Cu, Co, Fe, Hg, Ni, Zn, U	(Bakatula et al., 2014; Rai et al., 2008)
43	<i>Oocystis</i> sp. Nägeli ex A.Braun	Oocystaceae	Cd, Ni, Pb	(Karaca, 2008)
44	<i>Jaaginema angustissimum</i> (<i>Oscillatoria angustissima</i>) (West & G.S.West) Anagnostidis & Komárek	Synechococcales familia incertae sedis	Zn	(Ahuja et al., 1999)
45	<i>Jaaginema quadripunctulatum</i> (<i>Oscillatoria quadripunctulata</i>) (Brühl & Biswas) Anagnostidis & Komárek	Synechococcales familia incertae sedis	Cu, Pb, Co, Zn	(Ajayan et al., 2011)
46	<i>Oscillatoria limosa</i> C.Agardh ex Gomont	Oscillatoriaceae	Cu, Cd, Fe, Zn, Ni, Pb	(Atoku et al., 2021)

47	<i>Phormidium nigrum</i> (<i>Oscillatoria nigra</i>) (Vaucher ex Gomont) Anagnostidis & Komárek	Oscillatoriaceae	Cr, Fe, Ni	(Rai et al., 2008)
48	<i>Oscillatoria</i> sp. Vaucher ex Gomont	Oscillatoriaceae	Cd, Cr, Ca	(Bon et al., 2021; Jayashree et al., 2012; Mathimani et al., 2024; Shukla et al., 2012)
49	<i>Oscillatoria tenuis</i> C.Agardh ex Gomont	Oscillatoriaceae	As	(Huang et al., 2014)
50	<i>Padina</i> sp. Adanson	Dictyotaceae	U	(Khani, 2011)
51	<i>Parachlorella kessleri</i> (Fott & Nováková) Krienitz, E.H.Hegewald, Hepperle, V.Huss, T.Rohr & M.Wolf	Chlorellaceae	Cd, Cr	(Bauenova et al., 2021)
52	<i>Phormidium bohneri</i> Schmidle	Oscillatoriaceae	Cr, Fe, Ni	(Rai et al., 2008)
53	<i>Phormidium</i> sp. Kützing ex Gomont	Oscillatoriaceae	Cr, Ca	(Mathimani et al., 2024; Shukla et al., 2012)
54	<i>Pylaiella littoralis</i> (Linnaeus) Kjellman	Acinetosporaceae	Fe, Cu, Co, Cd, Cr, Zn, Al	(Carrilho and Gilbert, 2000)
55	<i>Tetraselmis (Platymonas) subcordiformis</i> (Wille) Butcher	Chlorodendraceae	Sr	(Mei et al., 2006)
56	<i>Porphyridium purpureum (cruentum)</i> (Bory) K.M.Drew & R.Ross	Porphyridiaceae	Cu, Cd, Cr, Pb, Hg, Ni	(Karaca, 2008; Soeprbowati and Hariyati, 2013; Zaib et al., 2016)
57	<i>Sargassum filipendula</i> C.Agardh	Sargassaceae	Cu, Cr, Cd, Ni, Pb, Zn, Ag, Fe, Mn	(Cardoso et al., 2016; Davis et al., 2000; Seepersaud et

				al., 2018; Verma et al., 2018, 2016)
58	<i>Sargassum fluitans</i> (Børgesen) Børgesen	Sargassaceae	Cd, Pb, Cu, Zn, Ni	(Fourest and Volesky, 1996; Kratochvil et al., 1995; Leusch et al., 1995; López-Miranda et al., 2020)
59	<i>Sargassum natans</i> (Linnaeus) Gaillon	Sargassaceae	Pb	(López-Miranda et al., 2020)
60	<i>Sargassum</i> sp. C.Agardh	Sargassaceae	Cd, Zn	(Esteves et al., 2000; Mahmood et al., 2017)
61	<i>Sargassum vulgare</i> C.Agardh	Sargassaceae	Cu, Cd, Zn, Pb, Ni, Mn, Fe	(Seepersaud et al., 2018)
62	<i>Tetradismus obliquus</i> (<i>Scenedesmus acutus</i>) (Turpin) M.J.Wynne	Scenedesmaceae	Cd, Cu, Zn, Cr, Pb	(Alayi et al., 2021; Monteiro et al., 2011, 2009; Omar, 2002; P.S et al., 2021; Purushanahalli Shivagangaiah et al., 2021; Zhang et al., 2016; Zhou et al., 2012)
63	<i>Desmodesmus</i> (<i>Scenedesmus</i>) <i>protuberans</i> (F.E.Fritsch & M.F.Rich) E.Hegewald	Scenedesmaceae	Cd, Pb, Ni	(Karaca, 2008)
64	<i>Desmodesmus communis</i> (<i>Scenedesmus quadricauda</i>) (E.Hegewald) E.Hegewald	Scenedesmaceae	Zn, Cr, Cu, Pb	(Kafil et al., 2022; Omar, 2002)

65	<i>Scenedesmus</i> sp. Meyen	Scenedesmaceae	As, Fe, Zn	(Ajayan et al., 2015; Arora et al., 2017; Bte Jais et al., 2015)
66	<i>Spirogyra hyalina</i> Cleve	Spirogyraceae	Cd, Hg, Pb, As, Co	(Kumar and Oommen, 2012)
67	<i>Spirogyra</i> sp. Link	Spirogyraceae	Fe, Cr, Cu, Ni, As, Cd, Pb, Mn, Se, Zn	(Abioye et al., 2020; Mane and Bhosle, 2012; Rai et al., 2008)
68	<i>Limnospira (Spirulina) platensis</i> (Gomont) K.R.S.Santos & Hentschke	Microcoleaceae	Cd, Cr, Cu, Co, Ni, Pb, Al, Fe, Zn, Sr, Ba	(Balaji et al., 2015, 2014; Diaconu et al., 2023; Kumar et al., 2020; Rangsayatorn et al., 2002; Zinicovscaia et al., 2018)
69	<i>Limnospira (Arthrospira) indica</i> (Desikachary & Jeejibai) Nowicka- Krawczyk, Mühlsteinová & Hauer	Microcoleaceae	Pb, Cr, Cd	(Balaji et al., 2014)
70	<i>Limnospira (Arthrospira) maxima</i> (Setchell & N.L.Gardner) Nowicka- Krawczyk, Mühlsteinová & Hauer	Microcoleaceae	Pb, Cr, Cd	(Balaji et al., 2014)
71	<i>Spirulina</i> sp. Turpin ex Gomont	Spirulinaceae	Cr, Cd, Pb, Mn, Se, Fe, Cu, Zn	(Hernández and Olguín, 2002; Mane and Bhosle, 2012)
72	<i>Synechococcus elongatus</i> (Nägeli) Nägeli	Synechococcaceae	Fe, Mn	(Gomes et al., 2021)
73	<i>Tetradesmus (Scenedesmus) incrassatulus</i> (Bohlin) M.J.Wynne	Scenedesmaceae	Cr, Cd, Cu	(Alayi et al., 2021; Peña-Castro et al., 2004)

74	<i>Tetraselmis indica</i> Arora & Anil	Chlorodendraceae	Mn, Pb, Al, Ca, Cd, Cu	(Amit et al., 2017)
75	<i>Trichormus variabilis</i> (Kützinger ex Bornet & Flahault) Komárek & Anagnostidis	Aphanizomenonaceae	Cd	(El-Hameed et al., 2021)
76	<i>Ulothrix</i> sp. Kützinger	Ulotrichaceae	Ni	(Rai et al., 2008)
77	<i>Ulva lactuca</i> Linnaeus	Ulvaceae	Cd, Cu, Cr, Fe, Zn, Mn, Ni, Pb	(Mofeed, 2017)
78	<i>Vaucheria cruciata</i> (<i>debaryana</i>) (Vaucher) De Candolle	Vaucheriaceae	Cd, Pb	(Khan et al., 2023)

isms (Alori et al., 2022). It usually included biological augmentation (bioaugmentation), during which some selected strains of microorganisms are added to the process to accelerate the breakdown of a pollutant (Herrero and Stuckey, 2015).

Algae species take the HMs by biosorption and bioaccumulation (Singhal et al., 2021). During biosorption, certain living/non-living microorganisms or biomass can passively concentrate and bind pollutants onto their cellular structure through the physiochemical process and immobilize them (Volesky and Holan, 1995). In other words, biosorption is the term used to describe the capacity of biological materials to ingest HMs physically or chemically from wastewater (Fard et al., 2011). While bioaccumulation is carried out in the following stages of biosorption and involves living organisms (Hlihor et al., 2017). Bioaccumulation and biosorption are subcategories of bioremediation. During biosorption, metals are retained through interactions with functional groups on the cell

surface (e.g., adsorption, ion exchange). This process can be affected by variables, including ionic strength, environmental acidity, biomass concentration, temperature, particle size, and other ions (Pagnanelli et al., 2003; Vilar et al., 2005). It can occur with both living and non-living biomass, as it does not depend on cell metabolism. In contrast, bioaccumulation involves both intra- and extracellular processes. Therefore, only live biomass can perform bioaccumulation (Coelho et al., 2015). Algae species often filter nutrients, heavy metals (depending on the species), and other minerals from wastewater through a combination of biosorption and their ability to absorb, adsorb, and bioaccumulate. Since these species need nutrients to grow, algae growth occurs as these elements are removed from wastewater. Some are absorbed by outer cells, while others are absorbed by inner cells (Bwapwa et al., 2017) (Fig. 2).

Most algae species (e.g., *Euglena* sp., *Scenedesmus* sp., *Oscillatoria* sp., *Chlorel-*

la sp.) absorb contaminants and immobilize them within their cell structure; these microalgae biomass can later be used as energy-enriched biomass for biofuel generation (Kandasamy et al., 2021).

In some strains, HMs or other nutrients with positive charges are clasped negatively charged groups (*e.g.*, —OH/ hydroxyl, —COOH/ carboxyl, —SH/ sulphhydryl, —NH₂/ amino, —PO₃H₂/ phosphoryl) on the surface layer of the cell wall (adsorption) (Spain et al., 2021). While in several microalgae, these pollutants are taken into the algae cell (absorption) (Gündoğdu and Türk Çulha, 2023). These algae accumulate HMs in their intercellular regions or their vacuoles (Torres, 2016). *Spirogyra* algal species had a removal efficiency of 20 mg/L Cu (II) (58–85%) at 30 minutes (Bishnoi et al., 2004). *Cladophora glomerata* and *Oedogonium rivulare* are among the species with the ability to remove Co, Pb, Ni, Mn, Cd, Cr, Cu, and Fe from contaminated water (Vymazal, 1984). *Ulothrix zonata* and *Turbinaria or-*

nata are also considered great adsorbents of HMs (Nuhoglu et al., 2002; Vijayaraghavan et al., 2005).

Factors affecting phycoremediation

Algae can remove HMs in a variety of ways. This process depends on the metal type, taxon, pH, light, and temperature (Mehta and Gaur, 2005; Novis and Harding, 2007). As algae are sensitive to light and temperature, the efficiency of phycoremediation can also be affected through different seasons. For example, the best time to remove HM sand contamination by algae is variable depending on the season (Brake et al., 2004; Elbaz-Poulichet, 2000). The strain of algae is also important in the process of phycoremediation. Some strains are more resistant to pollutants and have a higher ability to detoxify the HMs. An ecological study of soil in polluted sites with insecticides shows the replacement of sensitive species with resistant species (Megharaj et al., 1999). As previously mentioned, the non-living biomass of microalgae has the ability to adsorb pol-

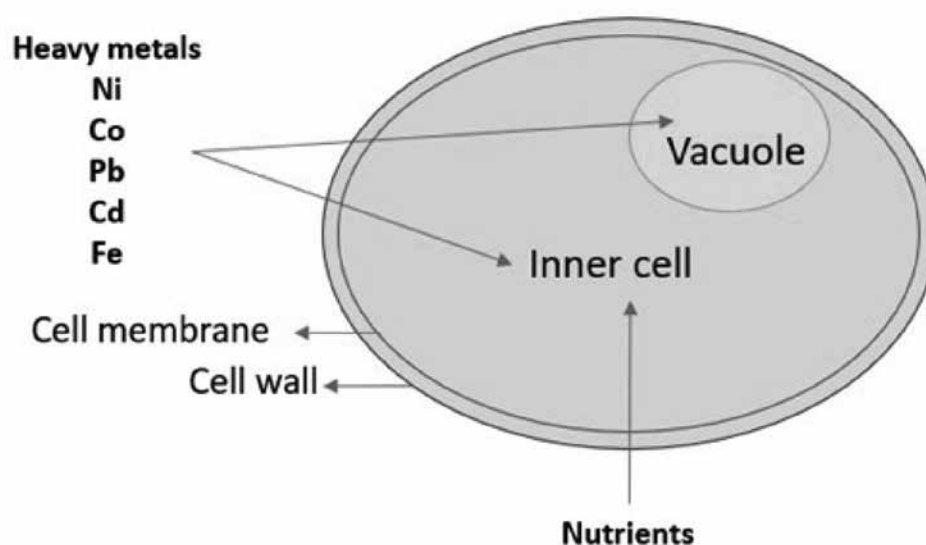


Fig. 2. A microalgae cell's absorption and adsorption scheme (modified from Kaplan, 2013)

lutants. It was revealed that the biomass of non-living algae adsorbs a higher amount of metals than that of living algae (Mehta and Gaur, 2005).

Methods to increase the efficiency of HM remediation by algae

To increase the efficiency of phycoremediation, some modern technologies have been developed in recent years. Chemical and molecular techniques are among the methods being used in this regard to manage algae to boost their productivity.

Transgenic algae to improve bioremediation

Genetic engineering for algae gene editing and gene silencing benefits various technologies and tools such as reporter genes, Cre-lox recombination, and CRISPR-Cas systems, modular cloning toolkits, regulatory elements, promoters, and vectors, restriction enzymes, and post-transcriptional gene silencing (PTGS) technologies (Fajardo et al., 2020). Data required for finding the appropriate genes to manipulate genetically is supplied by multi-omics approaches, including data of proteomics, transcriptomics, genomics, interactomics, and metabolomics, for various strains of algae, and is freely accessible on different online platforms (Ranjbar and Malcata, 2022) due to accelerated anthropogenic activities, and is nowadays, a matter of serious global concern. Removal of such inorganic pollutants from aquatic environments via biological processes has earned great popularity, for its cost-effectiveness and high efficiency, compared to conventional physicochemical methods. Among candidate organisms, microalgae offer several competitive advantages; phy-

coremediation has even been claimed as the next generation of wastewater treatment technologies. Furthermore, integration of microalgae-mediated wastewater treatment and bioenergy production adds favorably to the economic feasibility of the former process—with energy security coming along with environmental sustainability. However, poor biomass productivity under abiotic stress conditions has hindered the large-scale deployment of microalgae. Recent advances encompassing molecular tools for genome editing, together with the advent of multiomics technologies and computational approaches, have permitted the design of tailor-made microalgal cell factories, which encompass multiple beneficial traits, while circumventing those associated with the bioaccumulation of unfavorable chemicals. Previous studies unfolded several routes through which genetic engineering-mediated improvements appear feasible (encompassing sequestration/uptake capacity and specificity for heavy metals).

Transporters of HMs in algae cell membranes are important options in genetic engineering. These membrane proteins, which are responsible for the transportation and tolerance of metals, basically Co, Cd, Fe, Ni, Mn, and Zn, are known as metal-tolerance proteins (MTP) (Ram et al., 2019). Some species of microalgae, including *Microcystis aeruginosa*, *Spirulina* sp., *Synechococcus* sp., *Nostoc* sp., *Anabaena flos-aquae*, and *Fischerella*, carry MTP genes. These genes, which are involved in the regulation of metal ion storage, are expressed in response to higher concentrations of HMs like Cu (Kandasamy et al., 2021). Different

families of MTP genes are known in *Chlamydomonas*. More than eleven gene families are responsible for encoding the metal ion transporters (Rajamani et al., 2007). Up-regulation of *CRMTP4*, which encodes the metal transporter, enhances the tolerance of *Chlamydomonas reinhardtii* to the toxicity of Cd; these microalgae strains with upregulated *CRMTP4* had 2.81-3.06 times higher ability in bioaccumulation of Cd in comparison to wild *Chlamydomonas reinhardtii* (Ibuot et al., 2017).

However, the authors indicated that wastewater-adapted strains, *Parachlorella kessleri*, *Parachlorella hussii*, and *Jaagichlorella (Chlorella) luteoviridis*, had also higher tolerance to Cd, Zn, Al, and Cu than the wild strain of *Chlamydomonas reinhardtii*. These three microalgae also revealed higher tolerance and bioaccumulation of Cd than the upregulated *CRMTP4 Chlamydomonas reinhardtii*. This indicated clearly that the mechanisms of adapted strains, which can be attributed to their oxidative stress tolerance and upregulation of several genes, overcome the upregulation of a single MTP gene (Ibuot et al., 2017), and therefore, producing transgenic microalgae with multi-metal tolerance and absorption should be prioritized in genetic engineering of algae.

In *Auxenochlorella protothecoides*, high expression of metal transporter genes, the Nramp family, has been shown under Cd stress (Lu et al., 2019). These genes also play a role in Cd tolerance of *Chlamydomonas acidophila* (Puente-Sánchez et al., 2018). In *Chlamydomonas reinhardtii*, a member of the Nramp family, *DMT1*, is responsible for the transportation of Cd, Cu,

Fe, and Mn (Rosakis and Köster, 2005). Moreover, it seems that *MTP1* in *Chlamydomonas reinhardtii* encodes the vacuolar membrane protein which plays a critical role in detoxification of Cd and homeostasis of Zn (Blaby-Haas and Merchant, 2012). Phosphate transporters in *Microcystis aeruginosa* play a role in accumulation of arsenate (As) due to similar chemical structure of organic phosphate and As, lead to indiscrimination between these two elements (Wang et al., 2019).

Expression of *acr3* gene, which encodes protein ACR3 present in vacuole membrane of *Pteris vittata* and involved in bioaccumulation of As, in *Chlamydomonas reinhardtii* resulted in 1.5-3 times enhancement of As accumulation; the ability of this recombinant strain in bioaccumulation of As was even higher in environment with reduced phosphate (Ramírez-Rodríguez et al., 2019). Microalga *Euglena gracilis* exposed to Cd, Hg, and Pb revealed an enhancement in membrane transporter Major Facilitator Superfamily, P(1B)-type ATPases, Cd/Zn-transporting ATPase, as well as proteins participating in microalgae stress response and thiol-rich proteins which play an important role in metal chelation, at proteome level (Khatriwada et al., 2020). Cell surface engineering can also be employed to enhance the algae-based bioremediation of HMs. Transgenic *Chlamydomonas reinhardtii* due to plasma membrane-anchored metallothionein polymer expression revealed enhanced capacity for Hg (II) binding compared to wild strains (He et al., 2011).

It has also been shown that microalgae under HM stress upregulate particular HM-binding

organic molecules in order to reduce the HM toxicity through the formation of chelated forms (Balzano et al., 2020). Transformed *Chlamydomonas reinhardtii* with increased synthesis of cysteine (*HAL2* gene) revealed 5-times enhancement in metal binding capacity (Rajamani et al., 2007; Ranjbar and Malcata, 2022) due to accelerated anthropogenic activities, and is nowadays, a matter of serious global concern. Removal of such inorganic pollutants from aquatic environments via biological processes has earned great popularity, for its cost-effectiveness and high efficiency, compared to conventional physicochemical methods. Among candidate organisms, microalgae offer several competitive advantages; phycoremediation has even been claimed as the next generation of wastewater treatment technologies. Furthermore, integration of microalgae-mediated wastewater treatment and bioenergy production adds favorably to the economic feasibility of the former process—with energy security coming along with environmental sustainability. However, poor biomass productivity under abiotic stress conditions has hindered the large-scale deployment of microalgae. Recent advances encompassing molecular tools for genome editing, together with the advent of multiomics technologies and computational approaches, have permitted the design of tailor-made microalgal cell factories, which encompass multiple beneficial traits, while circumventing those associated with the bioaccumulation of unfavorable chemicals. Previous studies unfolded several routes through which genetic engineering-mediated improvements appear feasible (encompassing sequestration/uptake

capacity and specificity for heavy metals. Moreover, the engineering of microalgae to enhance the activity of particular enzymes to tolerate HM can be effective. For example, in *Chlorella vulgaris* chromate reductase play a role in the reducing toxic of Cr (VI) to the less dangerous trivalent chromium (Cr (III)). Therefore, it enhances the tolerance of microalgae cells against Cr toxicity (Yen et al., 2017).

Biochar addition for optimizing the phycoremediation

As biochar is enriched with nutritional components, a combination of biochar made from plant biomass with microalgae could aid in the cleanup of HMs and other hazardous materials. Microalgae may use biochar nutrients to boost their biomass. The bioremediation process is carried out simultaneously by biochar and metal-tolerant algae. This promising and long-term technique could result in more efficient phycoremediation with energy-containing biomass of microalgae. With the appropriate energy conversion method, this energy-enriched biomass of microalgae is able to generate a greater volume of ethanol (Anaé et al., 2021).

Biogenic nanomaterials generated from algae

Biogenic nanoparticles are those that are produced using biological organisms. Biologically produced nanoparticles have emerged as a viable substitute for chemically synthesized nanoparticles due to their nontoxicity. Several biogenic nanoparticles have been produced in recent years with possible applications in medicine and environmental cleanup. Biogenic nanoparticles

like palladium nanocrystals, nano-magnets, biogenic manganese oxide (BioMnOx), and biogenic iron species have been shown to be successful at removing a variety of micro-pollutants, HMs, refractory pollutants, and halogenated chemicals. Nano-bioremediation has the potential to be a more effective, safer, environmentally friendly, and cost-efficient technology, with a significant long-term impact on the field of environmental remediation (Kumari et al., 2019).

Algal nanocomposites reveal novel materials that mix algae-based polymers with nanoparticles. One of the main applications of these nanocomposites is in the remediation of wastewater. The application of alginate, derived from algae, as the base material in wastewater treatment is a green alternative to conventional fossil-fuel-based treatment methods (Lakshmi et al., 2023). Researchers have developed a *Fucus vesiculosus*-based sorbent for the effective removal of HMs, including Pb (II), Cd (II), Cu (II), and Zn (II) from polluted waters (Demey et al., 2018). Moreover, the ability of algal-made nanocomposites for the removal of Cr (VI) and iron compounds has been approved (Wu et al., 2018). In another research, a higher ability of *Sargassum glaucescens* and chitosan/polyvinyl alcohol (PVA) nano-fiber membrane at pH 6 for biosorption of Ni in a continuous system has been shown (Esmaeili and Aghababai Beni, 2018). The world nanomaterials market, including algal nanocomposites, reached 10.88 billion US dollars in 2022 and is projected to show a 14.8% growth by 2030 (Yuan et al., 2023).

Algae and bacterial consortia

Microalgae combine with other aerobic or

anaerobic microorganisms to form a microbial community. Compared with a single microorganism, a combination of algae and bacteria can work together to eliminate organic and inorganic pollutants. The combined use of microalgae and bacteria can be complementary and synergistic to obtain better pollutant degradation efficiency (Fu and Secundo, 2016). For instance, consortia of algae and bacteria mix revealed a significant removal rate of 92.6% for 1,2-dichloroethane from the petroleum industry (Alhajeri et al., 2024). On the one hand, algae photosynthesis produces oxygen, which is a key electron acceptor for heterotrophic bacteria to break down pollutants into organic matter. On the other side, bacteria provide carbon dioxide and other stimulating media to support the photosynthetic autotrophic growth of their partners (Subashchandrabose et al., 2011). The mixing of different strains, *i.e.*, algae-bacteria, can produce a synergistic effect, and the microbial population usually acts more effectively than a single strain or species. Some advantages of co-cultivation are the robustness to environmental fluctuations, the stability of the limbs, the ability to survive periods of nutrient limitation, to share metabolites, and resistance against other species. The self-oxidation of these natural systems that have been tested is beneficially used to remediate many pollutants (Muñoz and Guieysse, 2006). Compared with traditional engineering technology, it is more economically and technologically superior (Subashchandrabose et al., 2013). Contemporary molecular technology, combined with the careful selection of specific members of the microbial community, will

enable the creation of autonomous systems that serve the dual purpose of contaminant removal and metabolite production.

The bacteria-algae complex is effective in dealing with harmful pollutants, and their efficiency in the bioremediation of HMs has been established (Boivin et al., 2007). The normal growth and metabolism of algae require small amounts of various metals, but higher levels of the same metals are toxic. In this way, algae communities in symbiotic interactions can absorb and detoxify the metals. The process of detoxification involves physical or chemical adsorption, active absorption into the cell for a small amount, covalent bonding, ion exchange, surface precipitation, redox reaction, or cell surface crystallization (Muñoz and Guieysse, 2006; Subashchandrabose et al., 2013). Besides the HMs, the mentioned methods can be used by microalgae to degrade organic contaminants such as black oil, naphthalene, acetonitrile, phenol, thiocyanate, benzopyrene, azo compounds (Mahdavi et al., 2015; Muñoz and Guieysse, 2006; Ryu et al., 2015; Subashchandrabose et al., 2013), and toxic pesticides including methion, quinophos, methyl parathion, DDT, atrazine, and α -endosulfan (Subashchandrabose et al., 2013, 2011).

Compared with individual microorganisms, microalgae and bacterial consortia can effectively detoxify inorganic and organic contaminants and remove nutrients from wastewater. The resource competition and pollutant reduction cooperation between the two microbial associations will determine the success of the consortium project while harnessing the biotechnology potential of the partners (Subashchandrabose et

al., 2011).

Conclusion and perspectives

Bioremediation of polluted environments has attracted much attention during the last decades. As it is considered an eco-friendly and cost-effective method of treating contaminated water and soil, it has some advantages over other known existing techniques. Microalgae with an excessive tolerance to HMs and a high capacity for metal ion binding are the best accumulators of metals. Algae species such as *Chlorella*, *Spirulina*, *Spirogyra*, *Scenedesmus*, and many others are applied for the disposal of Cr, Cu, Ni, Cd, Hg, Sp, Pb, and other HM ions. Although using algae for bioremediation of HMs could encounter some problems, such as poor adaptability of exogenous microalgae with contaminated sites, and is affected by the intensity of light, operation time, and temperature, yet using various techniques, including ex-situ bioremediation and bioaugmentation, can help to manage these limitations. Moreover, some new methods and technologies have been employed to enhance the efficiency of phycoremediation. Biochar addition, applying genetically engineered and transgenic microalgae with MTP genes, and consortia of microalgae together or with other microorganisms, are among the new techniques that are rapidly growing to provide a greener world.

In spite of numerous advantages, some novel techniques encounter challenges, including scaling up. Most phycoremediation processes employing genetically engineered algae are still confined to laboratory settings (Pradhan et al., 2022). The main limitations

include low product yields and high cultivation costs (Wang et al., 2024). In order to address these challenges, it is necessary for future studies to focus on the expansion and development of universal cloning tool-kits and rapid expression kits, which enable gene editing tools to be applicable to a broad range of microalgae (Webster et al., 2024).

Algae-bacteria consortia have a notable advantage over other monoculture techniques in resistance to contamination (Naseema Rasheed et al., 2023). This feature makes them suitable for application in open ponds (Su et al., 2022). Moreover, the partnership offers practical benefits in harvesting the biomass due to enhanced flocculation efficiency when certain strains of bacteria are co-cultured with algae (Ravindran et al., 2016). Recent developments have focused on creating optimized consortia through careful selection of species and engineering. It has been shown that identification of the most effective combination, with some engineered consortia, achieves over 90% removal for various pollutants (Cai et al., 2024). These systems not only perform better in terms of pollutant removal but also generate valuable biomass that can be used for various applications (Navarro and Caipang, 2024; Torres et al., 2024).

The implementation of advanced algal bioremediation techniques remains primarily in developmental stages, with most successful applications in controlled conditions. A key challenge in scaling up these technologies is a requirement for a better understanding of how microalgae-microalgae or microalgae-bacteria co-culture perform

in open systems over a long time (Al-Jabri et al., 2020). The future success of these applications will depend on continued research to optimize performance and validate long-term effectiveness, particularly in outdoor conditions where environmental factors can significantly impact system performance (Al-Jabri et al., 2020). Natural symbiotic relationships between algae and native microorganisms show promise, particularly for water treatment applications, as these partnerships can effectively utilize carbon dioxide and minerals while producing oxygen without generating waste products (Touliabah et al., 2022). In Iran, there is inadequate information about the implementation of these methods. However, these approaches could be applicable in local environments.

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