



## **Potential of Algae in Bioremediation of Heavy Metals: A review**

**Meenakshi Sati<sup>1,\*</sup>, Megha Verma<sup>1</sup>, Megha Bora<sup>1</sup> and J.P.N. Rai<sup>1</sup>**

<sup>1</sup> Department of Environmental Sciences, College of Basic Sciences and Humanities, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

\*Correspondence: [minakshi088@gmail.com](mailto:minakshi088@gmail.com); Tel: 9536510504

### **ABSTRACT**

*Heavy metal pollution, a prevalent environmental problem worldwide, occurs directly by effluent out falls from industries, refineries and waste treatment plants and indirectly by the contaminants that enter the water supply from soils/ground water and from the atmosphere via rain water. Heavy metals are particularly hazardous because of their toxicity, tendency to bioaccumulate via food chain, and their abundance and persistence in the environment. Conventional methods for removing metals include chemical precipitation, chemical oxidation or reduction, ion exchange, electrochemical treatment, and membrane technologies, which may be ineffective or extremely expensive, especially when the metals are in diluted state. Microorganisms can retain relatively high quantities of metals by the process of bioremediation. The process of accumulation and adsorption of metals by algae involves adsorption onto the cell surface (wall, membrane or external polysaccharides) and binding to cytoplasmic ligands, phytochelatin and metallothioneins, and other intracellular molecules. The algal cell wall has many functional groups, such as hydroxyl (OH), phosphoryl (PO<sub>3</sub>O<sub>2</sub>), amino (NH<sub>2</sub>), carboxyl (COOH), sulphhydryl (SH), etc., which confer negative charge to the cell surface. Since metal ions in water are generally in the cationic form, they are adsorbed onto the cell surface. In this review a detailed investigation on biosorptive properties of algae involved in bioremediation process emphasizing the binding sites present and the detoxification mechanisms involved alongwith the physicochemical factors affecting sorption process are discussed.*

**Keywords:** Bioremediation, Heavy metals, Algae, Pollution

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### **INTRODUCTION**

Increasing industrial activities is the main culprit behind most environmental pollution problems and ecosystem damage, coming from the accumulation of pollutants such as toxic metals (chromium, copper, lead, cadmium, zinc, nickel, etc.) [1]. Mine draining, metal industries, petroleum refining, tanning, photographing processing and electroplating are some of the main sources of heavy metals [2]. In addition domestic effluents, landfill leachate, agricultural runoff, and acid rain also contribute to heavy metals in wastewaters [3]. Environmental contamination by these heavy metals is a serious problem due to their incremental accumulation in the food chain. Unlike most organic wastes and the microbial load in aquatic bodies, metal contaminants are not biodegradable, tending to accumulate in living organisms, thus becoming a permanent burden on ecosystems [4]. For the purpose of environmental protection, therefore relevant national and international environmental protection agencies have prepared guidelines and standards requiring the industrial discharges to be duly treated to minimum acceptable limits.

Techniques presently in existence for removal of heavy metals from contaminated waters include: reverse osmosis, electro dialysis, ultra filtration, ion-exchange and chemical precipitation. However, all these methods have disadvantages like incomplete metal removal, high reagent and energy requirements, generation of toxic sludge or other waste products that required careful disposal [5]. With increasing environmental awareness and legal constraints being imposed on discharge of effluents, a need for cost effective alternative technologies are essential. In this endeavor, microbial biomass has emerged as an option for developing economic and eco-friendly waste water treatment process [6].

### Effects of Heavy Metals

Trace amounts ( $\mu\text{g l}^{-1}$ ) of some metal ions such as copper, zinc, cobalt, iron, nickel are required by living organisms as cofactors for the enzymatic activities. However, heavy metal ion concentrations at ppm ( $\text{mg l}^{-1}$ ) level are known to be toxic to the organisms because of irreversible inhibition of many enzymes by the heavy metal ions. In view of the human health impacts, each metal imparts different effects and symptoms (Table 1).

**Table 1. Effects of heavy metals on human health**

Metal	Effects on human health
Zn	Irritability, muscular stiffness, loss of appetite and nausea
Cr	Cancer in the digestive tract and lungs
Mn	Neurotoxicity, low hemoglobin levels, and gastrointestinal accumulation
Cu	Intestinal distress, kidney damage and anemia
Pb	Anemia, encephalopathy, hepatitis and nephritic syndrome
Cd	Cancer, kidney damage, mucous membrane destruction, vomiting, diarrhea, bone damage, and itai-itai disease, as well as affect the production of progesterone and testosterone
Ni	Chronic lung and kidney problems, gastrointestinal distress, pulmonary fibrosis and skin dermatitis.
Hg	Nervous system deterioration, protoplasm poisoning

### Disadvantages of conventional methods of metal ion removal

Many procedures have been applied in order to remove heavy metals from aqueous streams. Among the most commonly used techniques are chemical precipitation, lime coagulation, ion exchange, reverse osmosis, electrodialysis, ultrafiltration and solvent extraction [7]. These classical or conventional techniques give rise to several problems such as unpredictable metal ions removal and generation of toxic sludge which are often difficult to dewater and require extreme caution in their disposal [8]. Besides that, most of these methods also present some limitations whereby they are only economically viable at high or moderate concentrations of metals but not at low concentrations, containing from 1 to 100 mg/L of dissolved metals [9,10]. Heavy metal removal by classical techniques involves expensive methodologies. These are due to high energy and reagent requirements. The above techniques can be summarized as expensive, not environment friendly and usually dependent on the concentration of the waste. Therefore, the search for efficient, eco-friendly and cost effective remedies for wastewater treatment has been initiated. In recent years, research attention has been focused on biological methods for the treatment of effluents, some of which are in the process of commercialization [11]. There are three principle advantages of biological technologies for the removal of pollutants; first, biological processes can be carried out in situ at the contaminated site; Second, bioprocess technologies are usually environmentally benign (no secondary pollution) and third, they are cost effective. Of the different biological methods, bioremediation have been demonstrated to possess good potential to replace conventional methods for the removal of metals [12,13,14].

### BIOREMEDIATION

Heavy metals are not biodegradable and tend to accumulate in living organisms. To curtail heavy metal pollution problems, many processes have been developed for the treatment of metal containing waste waters. Heavy metals showed to affect a wide range of microalgal cellular activities including cell viability and membrane structure and properties. Many chemical contaminants, including organochlorine compounds, herbicides, domestic and municipal wastes, petroleum products and heavy metals are now recognized to have adverse effects on ocean environments, even when released at low levels [15]. The need for economical, effective and safe methods for removing heavy metals from waste waters has resulted in the search for unconventional materials that may be useful in reducing the levels of heavy metals in the environment. In this light, biological materials have emerged as an ecofriendly and economic option. Bioremediation, which uses the ability of biological materials to remove and accumulate heavy metals from aqueous solutions, has received considerable attention in recent years because of few advantages compared to traditional methods. Bioremediation uses cheaper materials such as naturally abundant microorganisms and macro-organisms including algae, fungi, and bacteria [16].

#### Algae as Biosorbent

Various biomaterials have been studied for their biosorptive properties and different types of biomass have shown levels of metal uptake high enough to carry out further research [12]. Out of these, the most promising type of biosorbent studied is the algal biomass (Table 2). Algae are of special interest in development of new biosorbent material due to their ready availability in unlimited quantities in seas and oceans and high sorption capacity. Algae are encountered in every place where water is present, at least periodically. They inhabit both the aqueous environment and land. They live both in saltwater (saline, salty lakes, seas and oceans) and in freshwater (springs, rivers, ponds, lakes and swamps). Some species

are able to live on snow and ice, while others in hot springs (the highest temperature at which algae were found was 358.2 K). Algae display varied morphological structure of the thallus (single-celled species (*Ochromonas ludibunda*), species forming colonies (*Hydrurus foetidus*), and multi-celled organisms (*Ulva lactuca*), species with thread-like thallus (*Cladophora*) and ramified thallus (*Caulerpa prolifera*). They also vary in shape and size, from species as small as 1  $\mu\text{m}$  to large, leaf-like species that fix to surfaces using rhizoids (thread-like processes). In the trophic chain, algae are mostly autotrophic, although heterotrophic algae and algae that enter symbiotic relations with other organisms are also encountered. For instance, the symbiotic relations between algae and fungi take the form of lichens [17].

**Table 2. Some microalgal and macroalgal biosorbents**

Microalgal species	Metal sorbed	References
<i>Scenedesmus abundans</i>	K, Mg, Ca, Fe, Sr, Co, Cu, Mn, Ni, V, Zn, As, Cd, Mo, Pb, Se.	[18]
<i>Stigeoclonium tenue</i>	Cd, Pb, Zn	[19]
<i>Chlorella sorokiniana</i>	Ni	[20]
<i>Chlorella vulgaris</i>	Cd, Pb, Cu, Ag	[21]
<i>Chlorella miniata</i>	Cr(VI)	[22]
<i>Chlorococcum sp</i>	Cd, Pb, Cu, Ag	[21]
<i>Cyclotella cryptica</i>	Al, Zn, Pb, Cu, Cd	[23]
<i>Lyngbya taylorii</i>	Pb, Cd, Ni, Zn	[24]
<i>Porphyridium purpureum</i>	Al, Zn, Pb, Cu, Cd	[23]
<i>Scenedesmus quadricauda</i>	Cd, Pb, Cu, Ag	[21]
<i>Scenedesmus subspicatus</i>	Al, Zn, Pb, Cu, Cd	[23]
<i>Spirulina sp.</i>	Cd	[25]
<i>Spirulina platensis</i>	Cr	[26]
<i>Stichococcus bacillaris</i>	K, Mg, Ca, Fe, Sr, Co, Cu, Mn, Ni, V, Zn, As, Cd, Mo, Pb, Se.	[27]
Macroalgal species	Metal sorbed	References
<i>Spirogyra sp.</i>	Cr	[28]
<i>Cladophora fascicularis</i>	Pb	[29]
<i>Codium fragile</i>	Cd	[30]
<i>Fucus ceranoides</i>	Cd	[6]
<i>Fucus spiralis</i>	Cu	[31]
<i>Turbinaria conoides</i>	Pb	[32]
<i>Laminaria japonica</i>	Cd, Cu, Pb	[33,34]
<i>Gracilaria fischeri</i>	Cd, Cu	[35]
<i>Gracilaria sp.</i>	Pb, Cu, Cd, Zn, Ni	[36]
<i>Jania rubrens</i>	Pb	[37]
<i>Colpomenia sinuosa</i>	Cu, Ni	[38]
<i>Corallina officinalis</i>	Cd	[30]
<i>Durvillaea potatorum</i>	Cr	[39]
<i>Ulva sp</i>	Pb, Cu, Cd, Zn, Ni, Cu	[31]

### Bioremediation mechanism in algae

Elucidation of mechanisms active in metal biosorption is essential for successful exploitation of the phenomenon and for biosorbent regeneration in multiple re-useable cycles. The main uptake mechanisms by algae are: ion exchange between protons and heavy metal ions at the binding site or light metals and heavy metals, adsorption by physical forces, electrostatic interactions, chelation, complexation, microprecipitation (Table 3). Extracellular polysaccharides (such as alginates and fucoidans) are the main components of algal cell walls responsible for metal uptake. Sorption process can be classified in: chemical sorption (involve a chemical reaction) and physical sorption (sorption by physical forces - van der waals, electrostatic interaction). Theoretically, the type of sorption mechanism (physical /chemical) is given by the magnitude of  $\Delta H$  (enthalpy change) for a given sorption process calculated from sorption isotherm data obtained at different temperatures. Thermodynamically the heat of adsorption ( $\Delta H$ ), ranging from 0.5 to 5 kcal/mol (2.1–20.9 kJ/mol) gives physical adsorption mechanisms, and the value ranging from 5–100 kcal/mol (20.9–418.4 kJ/mol) gives chemical adsorption mechanisms [40].

**Table 3. Different mechanisms used by algae for heavy metal bioremediation**

Mechanism	Reference
Ion exchange	[41,42,43,44]
Coordination or complex formation	[45]
Microprecipitation	[46]
Chelation	[47,22]

### Bioaccumulation of Heavy Metals on Algae

Bioaccumulation normally means intracellular binding by a living organism [47]. Even though both living and dead biomass are capable of metal accumulation, there are some differences in the mechanisms involved depending on the extent of metabolic differences in the live algae. Heavy metal binding by living organism is generally known as bioaccumulation. Accumulation of heavy metals by living algae occurs in two phases: a rapid surface reaction followed by much slower metal uptake over a period of hours. The physical properties of alginate component in four different seaweeds (*Sargassum fluitans*, *Ascophyllum nodosum*, *Fucus vesiculosus* and *Laminaria japonica*) were characterized using potentiometric titration, carbon magnetic resonance, chemical analysis and viscosity measurements. The metal binding capacities were directly proportional to their respective carboxyl group content and electronegativity of the metal under investigation [48]. In bioaccumulation of heavy metals by living algal biomass, pH also plays an important role. The biosorption of  $\text{Cr}^{3+}$  by *Sargassum sp.* studied by [49] showed that pH has an important effect on  $\text{Cr}^{3+}$  biosorption capacity whereas biosorbent size does not affect the  $\text{Cr}^{3+}$  biosorption rate and capacity. The removal of  $\text{Cr}^{6+}$  by brown seaweed *Eclonia* was examined in the binary aqueous solution containing  $\text{Ni}^{2+}$ . The removal rate was unaffected by the presence of  $\text{Ni}^{2+}$ . The biosorption of  $\text{Cr}^{6+}$  from saline solutions on two strains of living *Dunaliella* algae were also tested under laboratory conditions as a function of pH, initial metal ion concentration and salt (NaCl) concentration in a batch system [50]. The sorption capacities of both sorbents for  $\text{Cr}^{6+}$  were obtained at pH 2.0 in the absence and in the presence of salt. [51] studied  $\text{Cd}^{2+}$  removal using *Cystoseira barbata* in batch, semi-batch and flow reactors. In batch and semi-batch tests, a local strain of *Cystoseira* algae removed 80-94% of the  $\text{Cd}^{2+}$  introduced.

Bioremediation has been attributed to different types of chemical groups such as hydroxyl, carbonyl, carboxyl, sulfhydryl, thioether, sulfonate, amine, imine, amide, imidazole, phosphonate and phosphodiester groups present in macromolecules in the cell wall of various microorganisms [52]. Table 4. gives an idea about various binding sites available on algae. The importance of any given group for biosorption of a certain metal by a certain biomass depends on factors such as: the number of sites in the biosorbent materials, the accessibility of the sites, the chemical state of the sites (i.e. availability), and affinity between site and metal [53]. [54] proposed that, for example, carboxylate, sulphate and amino groups may be responsible for metal binding by freshwater algae. The chelating ability of polysaccharides from freshwater alga *Chlorella* was connected to their content of uronic acids. Their carboxyl groups would be negatively charged and could bind metal ions [54,55]. [52] noticed a complete loss of available sulfhydryl groups (determined by polarimetric titration) after the binding of Au(III) to the freshwater algae *Chlorella*, thus giving evidence for involvement of this group. For brown marine alga it is assumed that alginates play a key role in metal-ion binding [47]. [56] investigated the binding of Hg, Cd, and Pb by alginic acid and *Sargassum fluitans* biomass before and after modification of the carboxyl groups using acidic methanol or propylene oxide.

**Table 4. Different binding sites present on algae [57]**

Group	Structural formula	Ligand atom	Occurrence in biomolecules*
Hydroxyl	-OH	O	PS, UA, SPS, AA, PP
Carboxyl(Ketone)	>C=O	O	Peptide bond
Carboxyl	-COOH	O	UA, AA
Sulfhydryl	-SH	S	AA
Thioether	>S	S	AA
Sulfonate	-SO <sub>2</sub> OH	O	SPS
Amine	-NH	N	Cto, AA
Secondary amine	>NH	N	Cti, PG, Peptide bond
Imine	=NH	N	AA
Amide	-CONH <sub>2</sub>	N	AA
Imidazole	-C <sub>3</sub> H <sub>4</sub> N <sub>2</sub>	N	AA
Phosphonate	C-PO(OH) <sub>2</sub>	O	PL
Phosphodiester	>PO(OH)	O	TA, LPS

(\*) PS-polysaccharides; UA-uronic acids; SPS-sulfated PS; Cto-chitosan; Cti-chitin; PG-peptidoglycan; AA-amino acids; TA-teichoic acid; PL-phospholipids; LPS-lipopolysaccharides; PP-polyhydroxypolyphenols

### 3. Heavy metal tolerance mechanism's in algae

Organisms respond to heavy metal stress using different defense systems (Table 5), such as exclusion, compartmentalization, making complexes and the synthesis of binding proteins such as metallothioneins (MTs) or phytochelatin (PCs) and translocate them into vacuoles [58]. Carboxylic and amino acids, such as citrate, malate, and oxalate, histidine (His) and nicotianamine (NA), and phosphate derivatives

(phytate) are potential ligands for heavy metals and are found to play a role in tolerance and detoxification [59,60]. The adsorption, phytosorption and affinity of algae for heavy metal cations in wastewater treatment because of its high negatively charged surface (cell wall components) have been acknowledged for a long time [59]. Two marine algae, *Thalassiosira weissflogii* and *Thalassiosira pseudonana*, produce phytochelatin in great amounts due to the higher activity of phytochelatin synthase, which has greater affinity for the glutathione substrate or metal ions [60,61,62]. Previously, [63], suggested that proline accumulation in green algae *Chlorella vulgaris* was highest in cells treated with Cu and Cr, founding a close link between the three processes of metal uptake, metal toxicity and proline accumulation and showing a protective effect of proline on metal toxicity through inhibition of lipid peroxidation.

**Table 5. Detoxification mechanisms of heavy metals by algae**

Metal	Detoxification mechanisms	References
Cd, Cu, Cr, Ag, Hg, Zn and Pb	Metallothioneins (MTs) and Phytochelatins (PCs).	[64,65,66]
Ni	Histidine	[67]
Pb, Cu, Cd, Zn, Ca	Cell wall components (Alginates and guluronic acid,sulfated polysaccharides and alginates)	[47,68]

### Metallothioneins for heavy metal Detoxification

During evolution, aquatic and terrestrial organisms have developed diverse strategies to maintain an equilibrated relation with heavy metal ions present and available in the surrounding medium. Cells face two tasks, the first is to select those heavy metals essential for growth and exclude those that are not, and the second to keep essential ions at optimal intracellular concentrations [69]. Land plants, aquatic plants and algae have all attracted considerable attention for the capacity to eliminate heavy metal. Much of the knowledge concerning algae is based in observations of higher plants. The research reviewed here reflects this fact but stresses important discoveries relating to microalgae, for example, the evidence of the ecological importance of algal mediated chelating mechanisms in real environments. Microalgae, related eukaryotic photosynthetic organisms, and some fungi have preferentially developed the production of peptides capable to bind heavy metals. These molecules, as organometallic complexes, are further partitioned inside vacuoles to facilitate appropriate control of the cytoplasmic concentration of heavy metal ions, thus preventing or neutralizing their potential toxic effect. In contrast to this mechanism used by eukaryotes, prokaryotic cells employ ATPconsuming efflux of heavy metals or enzymatic change of speciation to achieve detoxification [70]. The peptides discussed can be grouped into two categories:

1. The enzymatically synthesized short-chain polypeptides named phytochelatins (class III metallothioneins), found in higher plants, algae, and certain fungi.
2. The gene-encoded proteins; class II metallothioneins (identified in cyanobacteria, algae and higher plants), and class I metallothioneins found in most vertebrates, observed in *Neurospora* and *Agaricus bisporus* (not reported in algae) [71,72,73].

### Class III metallothionein (MtIII) in algae

MtIII biosynthesis can be induced by heavy metals such as Cd<sup>2+</sup>, Ag<sup>+</sup>, Bi<sup>3+</sup>, Pb<sup>2+</sup>, Zn<sup>2+</sup>, Cu<sup>2+</sup>, Hg<sup>2+</sup> and Au<sup>2+</sup> both in vivo and in vitro [74]. [75] found MtIII accumulation when *Stichococcus bacillaris* was exposed to As<sup>3+</sup>. In high concentrations of Cd<sup>2+</sup>, Pb<sup>2+</sup>, Ni<sup>2+</sup>, Zn<sup>2+</sup>, Co<sup>2+</sup>, Ag<sup>+</sup> and Hg<sup>2+</sup> occurring in non-contaminated natural waters, [76] found induction of MtIII synthesis in *Thalassiosira weissflogii*. [77] found the same pattern in the freshwater microalga *Scenedesmus subspicatus*. Some species and ecotypes of algae can live in the presence of toxic metal concentrations that are lethal for other species or populations. MtIII clearly can have an important role in metal detoxification. [78] reported sequestration of approximately 70% of cytosolic Cd<sup>2+</sup> by MtIII in *Chlamydomonas reinhardtii*. It has been found that MtIII synthesis is related to degree of pollution in an aquatic environment. [79] detected a MtIII production gradient in the phytoplankton of a bay receiving anthropogenic sources of heavy metals, with higher MtIII production in zones nearer to the coast, which were likely to contained higher metal concentrations. [80] suggested that MtIII in algae could play a role not only in detoxification of heavy metal, but also in mitigation of oxidative stress. For example, [81] working with *Phaeodactylum tricornutum* and Cu<sup>2+</sup> found that some metal-free MtIII was present in cell extracts. They made the hypothesis that this could represent an oxidized form of MtIII that had been participated to scavenge reactive oxygen species.

[82] demonstrated that MtIII are synthesized by the enzyme, phytochelatin synthase (PCS), which is a c-glutamylcysteine dipeptidyl transpeptidase (E.C. 2.3.2.15) The fact that PCS activity in vitro tended to stop when heavy metal ions are removed and that in vivo the addition of a wide variety of heavy metal

ions activates MtIII synthesis, lead to the proposal that PCS are heavy-metal activated enzyme [83,65]. Cysteine is part of the MtIII chelating core and is an activator of PCS [84], so its upstream synthesis also seems important for the production of MtIII and maybe, the restrictive factor for the construction of new phenotypes appropriate for phytoremediation. [85] overexpressed O-acetylserine (thiol) lyase in *A. thaliana*. This enzyme is responsible for the final synthesis of cysteine. They found that the plant could grow in higher concentrations of Cd<sup>2+</sup> and accumulated more metal in the leaves. Sulfide ions (S<sub>2</sub><sup>-</sup>) are also present in metal–MtIII complexes [86]. This ions improves the stabilization of metal–MtIII compounds [87], in consequence, detoxification is also improved [88].

#### **Sequestration of heavy metals into the Vacuole and Mitochondria of algae**

In the green algae, *D. bioculata*, electron dense materials inside the vacuoles, which contained cadmium and sulfur in a ratio between 2 and 2.4, were detected when exposed to 100 mg L<sup>-1</sup> of Cd<sup>2+</sup> [89]. The green alga *T. suecica* exposed to Cd<sup>2+</sup> showed the metal accumulation in the cell wall and intracellular organelle [90]. In the diatom, *S. costatum*, accumulation of Cd<sup>2+</sup> and Cu<sup>2+</sup> in vacuole of cells was detected when grown in the presence of these metals. Again a predominant element in the inclusions was sulfur in a sulfur/metal ratio of 1.5 [91]. *Euglena gracilis* is a photosynthetic protist with high tolerance to Cd<sup>2+</sup> and high Cd<sup>2+</sup> accumulating capacity. This organism does not possess a specialized reservoir organelle such as a plant-like vacuole [92]. [93] working with *E. gracilis*, found that more than 60% of the accumulated Cd<sup>2+</sup> resides inside the chloroplast. This was correlated with an 4.4-times increase in more thiol-compounds and sulfide, compared to a control chloroplast. In a Cd<sup>2+</sup> treated chloroplast a significantly higher amount of MtIII was found, and glutathione represented the 66% of the total organic thiol content. [94] working with Hg<sup>2+</sup> pretreated heterotrophic cell of *E. gracilis* exposed to Cd<sup>2+</sup>, found that 79% of the total accumulated metal was in the mitochondria. They also found a remarkable increased in the Cys and glutathione concentration in Cd<sup>2+</sup> treated cells. The amount of MtIII in mitochondria was around 17% of the total MtIII found in treated cells.

#### **The Effect of Physicochemical Factors on the Sorption Properties of Algae**

The efficiency of heavy-metal sorption by algae used for the purposes of the *in situ* studies depends on the abiotic factors, *eg* the temperature, pH, the intensity of the photosynthetically active light as well as the presence of other ions and anions which cause, *eg* complexing of the metal ions (chlorides and humus substances) or development of difficult to dissolve metal compounds (phosphates). The biotic factors, for instance organic matter suspended in water through the surface sorption processes, decrease the equilibrium concentration of metals in water [95]. Due to the complexity of the aqueous environment as well as the interactions among various factors and elements of the ecosystem it is difficult to estimate, under natural conditions, the effect of the bioaccessible forms of heavy metals on the algae cells. The assessment of the effect of physiochemical factors on the sorption properties of different algae species was carried out. It was observed that changes in pH of Cu<sup>2+</sup> and Pb<sup>2+</sup> salt solutions (analyte concentration in solution was 100 mg/dm<sup>3</sup>) in the range 5.8 to 8.5 did not have any effect on the concentration of the ions accumulated in the alga *Ecklonia maxima*. Lack of increase in copper and lead concentrations in algae may have been caused by precipitation of the insoluble hydroxides from the solution and at the same time a limitation of their sorption by the thalli [96]. A decrease of solution pH (pH < 4) reduced the sorption of the analysed heavy metals. This outcome was induced by, *eg* competitiveness of H<sup>+</sup> ions in relation to Cu<sup>2+</sup> and Pb<sup>2+</sup> ions and a decrease in the number of negative charges on the surface of cell walls, which depends on the dissociation degree of functional groups, *eg* the carboxyl and amine groups. Together with the decrease in pH, the dissociation of carboxyl group of amino acids decreases. Under such conditions, a strong protonation of the amine group to NH<sup>3+</sup> takes place, which allows for a conclusion that in a strongly acid environment the amino acid particles are present in the form of a positive ion. Biosorbents (DP95Ca and ER95Ca) developed on the basis of the marine alga *Durvillaea potatorum* and *Ecklonia radiata* sorbed 90% of the determined metal ions at pH = 4.5. A decrease in pH (pH < 1) caused a decrease of the concentration of the absorbed metal ions [97]. It was observed that the alga *Ulothrix zonata* sorbs the most Cu<sup>2+</sup> ions at pH = 4.5 [98] and the algae *Spirogyra* sp., *Chlorella vulgaris*, *Scenedesmus obliquus* and *Synechocystis* sp. at pH = 5.0 [99].

The cations naturally present in the algae environment, *eg* Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup> and Ca<sup>2+</sup> also influence heavy-metal cation sorption by their thalli. The laboratory tests revealed a competitive sorption of Na<sup>+</sup> ions (introduced to the solution in the form of NaCl of the concentration = 1M) in relation to Hg<sup>2+</sup> ions (reduction of Hg<sup>2+</sup> sorption by 80%) by the alga *Cystoseira baccata*. Mercury concentration in the initial solutions was 500 and 1000 mg/dm<sup>3</sup>. Sodium and potassium ions introduced to the solution in the form of NaNO<sub>3</sub> and KNO<sub>3</sub> led to the increase of concentration of the absorbed mercury ions. The authors did not observe any effect of Ca<sup>2+</sup>, Mg<sup>2+</sup>, Zn<sup>2+</sup>, Cd<sup>2+</sup>, Pb<sup>2+</sup> and Cu<sup>2+</sup> ions on the sorption of Hg<sup>2+</sup> by the alga *Cystoseira baccata* [6].

The presence of  $K^+$ ,  $Mg^{2+}$  and  $Ca^{2+}$  ions in the saline solution of  $Cu^{2+}$  led to the decrease of concentration in the absorbed metal ions by the alga *Padina* sp. by 4, 11 and 13%, respectively. Sodium ions introduced to the copper saline solution in the form of NaOH did not have any effect on sorption of  $Cu^{2+}$  ions [100].  $Na^+$  and  $K^+$  ions (concentrations 0-10 mM) did not have any effect on sorption of  $Pb^{2+}$  and  $Cu^{2+}$  by the alga *Ecklonia radiata*, while  $Ca^{2+}$  and  $Mg^{2+}$  ions lead to a 10-18 and 5-10% decrease of sorbed metal ions in thalli respectively, depending on the introduced concentration of  $Ca^{2+}$  and  $Mg^{2+}$  (0-10 mM) [101]. Experimental results of sorption of  $Cr^{3+}$  ions by the algae *Spirogyra condensata* and *Rhizoclonium hieroglyphicum* indicate a competitive sorption of other metal ions. It was observed that enriching the chromium-ion solution with additional ions of  $Cu^{2+}$  and  $Pb^{2+}$  led to the reduction of chromium ions sorption by 38 and 36%, respectively [102]. Reduction of copper sorption from solution by the alga *Spirogyra neglecta* due to lead ions was also observed. A reverse phenomenon was also observed: copper limited lead sorption, however, the competitiveness of sorption was changing in the column  $Pb > Cu$  [103].

Anions like chloride, sulphates, phosphates and nitrate also influence the sorption properties of algae. The effect of chlorides, sulphates and nitrates on the  $Cr^{6+}$  ions sorption was studied (initial solution concentrations were 0.4-2.9 mmol/dm<sup>3</sup>). It was observed that anions ( $NO_3^-$ ,  $Cl^-$  and  $SO_4^{2-}$  conc- 10 mmol/dm<sup>3</sup>) decrease the concentration of the chromium ions absorbed by the thalli of *Cladophora albida* by 1.96% nitrates, by 6.63% chlorides and by 4.25% sulphates, respectively [104].

Temperature has a significant impact on various physical, biological and chemical processes that take place in an organism. For instance, it influences the viscosity, diffusion, osmosis, transport of mass and electrical charges, metabolic processes, as well as the stability of macromolecule structures of living organisms [105]. Various species of blue-green algae prosper in the temperatures between 303-308 K, and golden, red and green algae in the moderate temperatures of 288-298 K. Temperature increase within the range of 298-318 K led to the increase of concentration in the absorbed  $Pb^{2+}$  ions by the algae *Oedogonium* sp., *Nostoc* sp. and *Spirogyra* sp. At the temperature equaling 298 K, in the analyzed algae 144.92, 93.46 and 96.4 mg of Pb/g dm were found, respectively. Temperature increase to 318 K caused the increase of concentration of the absorbed  $Pb^{2+}$  ions up to 169.49, 106.38 and 104 mg/g dm, respectively. The increase of metal-ion biosorption along with the temperature increase suggests that the process is of an endothermic character. Temperature increase may lead to, among others, the increase of dissociation degree of functional groups of the algae cell walls [106]. On the other hand, the  $Ni^{2+}$  ion sorption by the alga *Oedogonium hatei* reveals an exothermic character. Temperature increase in the range between 289-318 K led to the decrease of metal-ion concentration by algae (298 K - 42 mg Ni/g dm, 318 K - 37.3 mg Ni/g dm). In this case, temperature increase may have damaged the active centers responsible for nickel sorption. The optimal temperature chosen by the authors for the purpose of conducting the processes of heavy-metal sorption by the algae *Oedogonium* sp., *Nostoc* sp. and *Spirogyra* sp. was 298 K [107].

#### Desorption of Heavy-Metal Ions from Algae

Cation-exchange properties of algae are connected, among others, with their cellular structures created by, eg alginic acid salts (alginates) [47,108,25,13]. Laboratory tests revealed that cation desorption by algae cellular structures may be performed with, eg mineral acids: HCl and  $HNO_3$  [103,109] salt - NaCl,  $Ca(NO_3)_2$  [110], as well as the chelating complexes: sodium salt of ethylenediaminetetraacetic acid  $Na_2EDTA$  (often referred to as EDTA) [111,112]. Applying the hydrochloric acid of 0.1 M concentration, 80-85% of copper ions and approx. 90% of lead ions were desorbed by the thalli of *Spirogyra neglecta* [113]. During desorption (0.1 M HCl) of heavy metals ( $Hg^{2+}$ ,  $Cd^{2+}$  and  $Pb^{2+}$ ) by the alga *Chlamydomonas reinhardtii*, the authors obtained approx. 98% of process capacity [111]. Using mineral acids ( $HNO_3$  and HCl) with concentrations of 0.05 M, during the first 2 min of the process approx.  $92 \pm 5\%$  of copper ions were extracted by the algae *Ulva fasciata* and *Sargassum* sp. [114]. Application of 0.1 M of HCl solution (process time - 15 min) induced 95.3% of metal desorption ( $Cu^{2+}$  concentration in algae - 133.3 mg/g dm) by the algae. NaCl solution and water desorbed  $Cu^{2+}$  from the biosorbent in 8.6 and 4.4%, respectively [115].

In the process of  $Pb^{2+}$  ions desorption by the thalli of *Sargassum* sp., a 95% capacity was attained with 0.1 M solution of  $Na_2EDTA$ . This fact may be explained by a high value of stability constant of the Pb(II)-EDTA complex which facilitates desorption of lead ions by the biomass [116]. Using 0.1 M of HCl for  $Pb^{2+}$  ions desorption by the thalli of *Oedogonium* sp. and *Nostoc* sp. led to the removal of metal from the biosorbent in approx. 90%. It was observed that carrying out the sorption-desorption process five times causes a 5% decrease in sorption capacity by the studied algae [106]. On the basis of the analysed results it can be concluded that algae may be repeatedly used as biosorbent in waste water treatment.

## CONCLUSION

The indiscriminate release of hazardous pollutants (including heavy metals, such as cadmium (Cd), chromium (Cr), lead (Pb), mercury (Hg), etc.) in the human environment pose a major threat to all kinds of organisms inhabiting aquatic as well as terrestrial ecosystems. The application of bioremediation technologies has emerged as the most promising techniques to mitigate this problem. Of the microorganism studied, algae are gaining increasing attention, due to their easy availability and cost effective nature. The development of bioremediation processes using algal biomass requires further investigation, with particular attention to: selectivity of algal species, regeneration and reusability of algal biomass, immobilization matrices for microalgae, simulation and modelling of processes. These challenges can be met only by a multidisciplinary approach involving phycology with microbiology, biochemistry, chemical and environmental engineering, process engineering, physical chemistry and analytical chemistry to optimize and scale up the applications.

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## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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