

*Chapter 4*

## **EFFECT OF SEAWEEDS IN PHYTO-REMEDIATION**

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### **ABSTRACT**

Heavy metals and organic pollutants are introduced into the aquatic ecosystems as a result of human activities involving agricultural uses, industrial discharges, domestic effluents and agricultural runoff. These contaminants such as herbicides, pesticides, nitrogen and phosphate fertilizers, heavy metals... etc., have negative impacts on both the stability of the natural aquatic environment (intensification of eutrophication, contamination and disappearance of certain animal and plant species...) and can cause adverse effects on human health. Recently there has been an increasing interest in using seaweeds for water quality assessment and for removal of heavy metals and organic pollutants. In this review, we will discuss the use of macroalgae as bioindicators for monitoring and protecting aquatic environments and different mechanisms used by these seaweeds for metal accumulation and detoxification.

**Keywords:** biosorption, bioaccumulation, phytoremediation, seaweeds

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## 1. INTRODUCTION

The accumulation of organic pollutants (pesticides, PCBs, DDT...) and heavy metals (Cd, Pb, Se, As...) in the aquatic systems can cause serious problems on environment and organisms affecting negatively the stability of many aquatic ecosystems and can also cause difficulties for animals and human health [1]. Although some metals are necessary for biological processes, all of them are toxic at high concentrations. This is due to their oxidative capacity to form free radicals and their ability to replace essential metals in enzymes, interrupting their normal activity [2]. Other metals are not essential and accumulate in different organisms and because of this they are toxic even at low concentrations. Mercury, chromium, lead, arsenic, copper, cadmium, cobalt, zinc, nickel, beryllium, manganese and tin are the most toxic heavy metals according to the United States Environmental Protection Agency (EPA) [3]. Many aquatic ecosystems have been subjected to industrial waste discharge. Domestic and agricultural pollution generating both organic and inorganic contamination, such as pesticides and heavy metals, are leading to widespread contamination of both surface and groundwater by runoff. Metals are introduced into the aquatic ecosystems as a result of weathering of soil and rocks, from volcanic eruptions and from a variety of human activities involving mining, processing and use of metals and/or substances containing metal contaminants [4]. These heavy metals may also be derived from remobilization from natural soils due to the changes in local redox conditions and the corrosion of subsurface engineering structures due to prolonged submergence under acidic groundwater [5]. Studying the bioavailability and origin of heavy metals from the Nador lagoon sediments, González et al. [6] found that the most important trace-element anomalies (As, Cd, Co, Cu, Mn, Pb, Zn) were found, mainly around industry and old mining activities. Industrial activity has led to very high heavy metal concentrations on the environment, which are in general 100–1000 fold higher than those in the Earth's crust, and locally, living organisms can be exposed to even higher levels [7]. In a river polluted by base-metal mining, cadmium was the most mobile and potentially bioavailable metal and was primarily scavenged by non-detrital carbonate minerals, organic matter, and iron-manganese oxide minerals [8]. Although mercury is a naturally occurring element and it was always present in the environment, global human activity has led to a significant increase of mercury released into the atmosphere, aquatic environment and land [9]. Wang et al. [10] suggested that the most important anthropogenic sources of mercury pollution in aquatic environment are

atmospheric deposition, urban discharges, agricultural material runoff, mining, fossil fuel use and industrial discharges, burning of coal, and pharmaceutical production [10]. In order to control heavy metal levels before they are released into the environment, the treatment of the contaminated wastewaters is of great importance since heavy metal ions accumulate in living species with a permanent toxic and carcinogenic effect [11, 12]. The most common treatment processes used include chemical precipitation, oxidation/reduction, ion exchange, membrane technologies, especially reverse osmosis, and solvent extraction. Each process presents advantages, disadvantages and ranges of applications depending on the metal ion, initial concentration, flow rate or raw water quality [13]. Martínez-Jerónimo et al. [14] suggested that chemical contaminants present in the aquatic ecosystem may be immobilized and accumulated in sediments or may be subject to transformation and activation processes [14]. Depending on biogeochemical processes, many organic pollutants like hydrocarbons are involved in adsorption, desorption and transformation processes and can be made available to benthic organisms as well as organisms in the water column through the sediment–water interface [15].

Physicochemical processes, conventionally used for metal removal, often have high operating costs, generate large amounts of sludge which require a proper disposal, or are ineffective when aim is to achieve very low residual levels [3, 16].

Relatively recently, there has been increasing interest on the use of bioremediation as the most desirable technology which uses seaweeds and other organisms for removal of environmental pollutants or detoxification to make them harmless [1, 17, 18]. Seaweeds can eliminate heavy metals by two processes: bioaccumulation and biosorption. Biosorption is a term that describes the removal of heavy metals by the passive binding to non-living biomass from an aqueous solution; however, the bioaccumulation describes an active process whereby removal of metals requires the metabolic activity of a living [19]. Investigation on organic xenobiotics bioaccumulation/biodegradation in green algae is of great importance from environmental point of view because widespread distribution of these compounds in agricultural areas has become one of the major problems in aquatic ecosystem [20]. Some algae and microorganisms have developed various strategies for their survival in heavy metal-polluted habitats, these organisms are known to develop and adopt different detoxifying mechanisms such as biosorption, bioaccumulation, biotransformation and biomineralization, which can be exploited for bioremediation either *ex situ* or *in situ* [21-24]. Biosorption may be simply

defined as the removal of substances from solution by biological material. Such substances can be organic and inorganic, and in soluble or insoluble forms [25]. Biosorption is a physico-chemical process and includes such mechanisms as absorption, adsorption, ion exchange, surface complexation and precipitation.

This chapter will highlight our current understanding on the involvement of seaweed in bioremediation of heavy metals and different strategies used by these species for metal accumulation and detoxification.

## **2. MECHANISMS OF METAL ACCUMULATION AND DETOXIFICATION**

### **2.1. Cell Wall Adsorption**

There are several chemical groups that could contribute to the metals acquisition by biomass: acetamido groups of chitin, structural polysaccharides of fungi, amino and phosphate groups in nucleic acids, amino, amido, sulfhydryl, and carboxyl groups in proteins, hydroxyls in polysaccharides, and mainly carboxyls and sulfates in the polysaccharides of marine algae that belong to divisions Phaeophyta, Rhodophyta, and Chlorophyta [26].

Algae have been used extensively as biosorbent material more than other kinds of biomass [27, 28]. Their ability focused on the composition of cell wall which includes molecules such as chitin, polysaccharides, proteins and lipids. These molecules have different groups such as phenolic, hydroxyl and carboxyl, which can form complexes with heavy metals. Previously, Wang et al. [29] suggested that polysaccharides such as cellulose, chitin, and alginates that are constituents of cell walls of fungi and algae participate in capturing metals. Stary and Kratzer [30] reported that the algae cell wall behaves like a weak acidic cation exchanger containing various cell wall ligands with different exchange capacities.

The cell walls of seaweeds (Phaeophyta, Rhodophyta and many Chlorophyta) are composed of at least two different layers (Figure 1). The innermost layer consists of a microfibrillar skeleton that imparts rigidity to the wall [19]. The outer layer is an amorphous embedding matrix [32, 33]. Takeda and Hirokawa [34] demonstrated that the cell wall of the green algae *Chlorella ellipsoidea* was composed of two major constituents: alkali-soluble hemicellulose and alkali insoluble rigid wall. The former was composed of

neutral sugars, rhanmose, xylose, arabinose, mannose and glucose, and the latter had glucosamine as a main constituent.

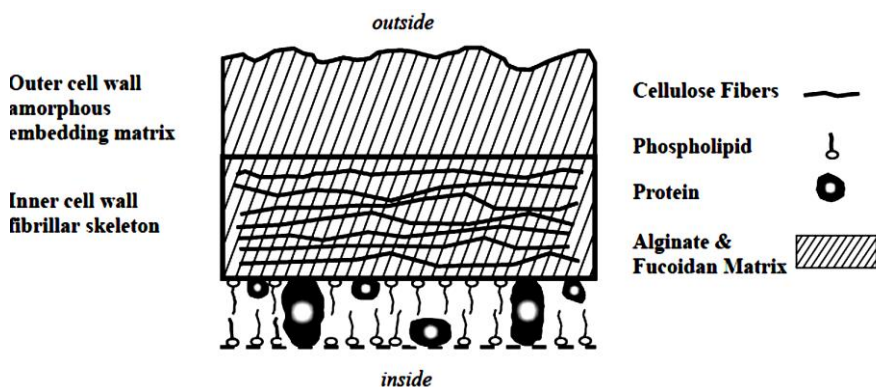


Figure 1. Cell wall structure in the brown algae [31].

The most common fibrillar skeleton material is cellulose which imparts rigidity to the cell wall [35]. It can be replaced by xylan in the Chlorophyta and Rhodophyta in addition to the mannan in the chlorophyta [19]. Besides cellulose, red and green algae contain, respectively, agar and carragenates, rich in sulfated polysaccharides, and glycoproteins, which comprise amino, carboxyl, sulfate and hydroxyl groups [36].

Lahaye and Robic [37] reported that among the polymers synthesized by the green seaweed *Ulva* and *Enteromorpha* cell wall polysaccharides represent around 38-54% of the dry algal matter. These include four polysaccharide families in *Ulva* sp.: two major ones, the water-soluble ulvan and insoluble cellulose, and two minor ones, a peculiar alkali-soluble linear xyloglucan and a glucuronan (Figure 2). The carboxylic and sulphate groups of these polysaccharids have been identified as the main metals equestering functional ionic groups in marine algal cell wall [38].

Davis et al. [19] suggested that the phaeophyta algal embedding matrix is predominately alginic acid, with smaller amounts of the sulphated polysaccharide fucoidan, while the Rhodophyta contain a number of sulphated galactan (e.g., agar, carrageenan, porphyran, etc.). Both the Pheophyta and Rhodophyta contain the largest amount of amorphous embedding matrix polysaccharides making them potentially excellent materials for heavy metal binding.

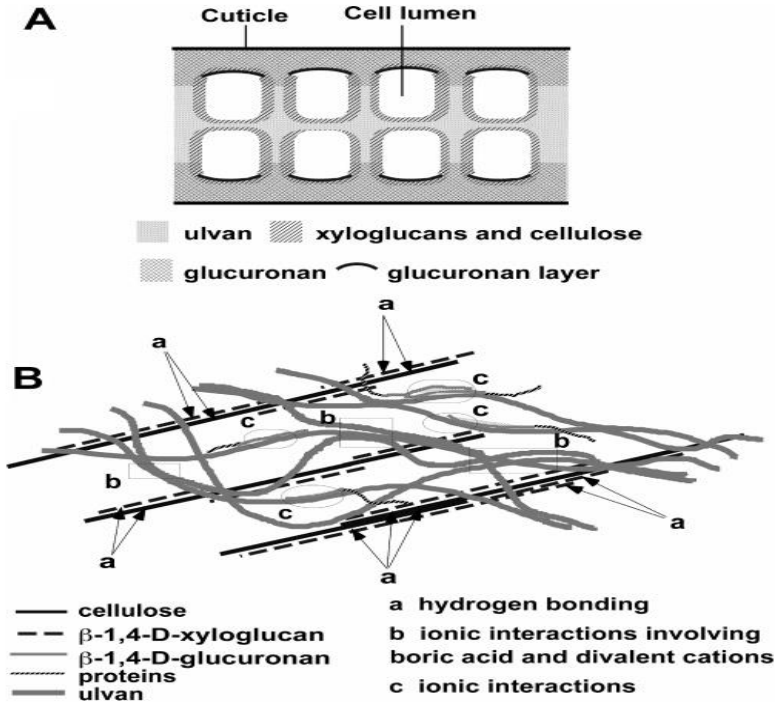


Figure 2. Distribution of the different *Ulva* sp. cell wall polysaccharides in a schematic cross section of a thallus (A) and proposed associations between the different cell wall polysaccharides (B) [37].

Poly-anionic polysaccharides of the red seaweed, associated with the cell walls and intercellular spaces, sequester cations through an ion exchange mechanism, thus partially preventing entry of metal ions into cells [39-41]. The brown algae have proven to be the most effective and promising substrates for the adsorption of heavy metals. The properties of cell wall constituents, such as alginate and fucoidan, are chiefly responsible for heavy metal chelation [19]. Carboxyl and sulfate are the predominant functional groups in brown algae cell walls, which are mainly composed by cellulose, alginic acid and sulfated polysaccharides [36].

## 2.2. Vacuolar Compartmentation

The vacuole is a suitable storage reservoir for excessively accumulated heavy metals [42]. Recently, Seth et al. [43] reported that translocation and

storage of metals into vacuoles are an important phenomenon found in non-accumulators where it is an efficient defence strategy against elevated metal concentrations.

Suresh and Ravishankar [44] reported that algae have the ability to hyperaccumulate various heavy metals by the action of phytochelatins and metallothioneins forming complexes with heavy metals and translocate them into vacuoles. Hall [45] suggested that the efflux of ions at the plasma membrane or transport into the vacuole by tonoplast-located transporters are the two ways of reducing levels of toxic metals in the cytosol and so are potentially important mechanisms for heavy metal tolerance. The objective of this compartmentation is to remove the metals from the cytosol or other cellular compartments where sensitive metabolic activity takes place [46-48]. Therefore, the central vacuole seems to be a suitable storage reservoir for excessively accumulated heavy metals [42]. Volland et al. [49] reported that  $Al_2(SO_4)_3$  treatment resulted in an increase of the number of vacuoles as a mechanism for the accumulation and retention of heavy metals.

Cobbett and Goldsbrough [50] showed that the potential toxicity of accumulated metals can be decreased as a result of the formation and subsequent sequestration of “metal-phytochelatin complexes” in vacuoles *via* transport across the tonoplast. Similarly, Heuillet et al. [51] suggested that the metal–metallothionein complex ends up in the vacuole of the cell. This was observed in the microalga *Dunaliella bioculata*. The compartmentalization of Zn in vacuoles seems to be a general crucial detoxification mechanism for cells [52, 53]. In *Cardaminopsis halleri*, a heavy-metal-tolerant plant, the vacuole is the main depot for Zn, where it is stored as zinc silicate [54]. Increased uptake of metals has been achieved in several examples using transporters located in the tonoplast that sequester metals into the vacuole [55-57].

Kakinuma et al. [58] reported that the accumulation of most of the metal ions is driven by the electrochemical potential by electrogenic proton influxes *via* the vacuolar  $H^+$ -ATPase. Cd is transported across the tonoplast by a  $Cd^{2+}/H^+$  antiport mechanism [59]. Rahman and Hassler [60] showed that the Phytochelatins build a complex with arsenite ( $As(III)$ -PC) and are sequestered into vacuoles through the activity of ATP binding cassette (ABC) transporters, being finally excreted from the cells. Song et al. [61] suggested that essential metal ions, such as Zn(II), Cu(II), and Mn(II), can be transported into vacuoles as forms of “PC2-metal complexes” through the putative ABC transporter(s).

## 2.3. Intracellular Sequestration

To maintain low concentrations of free metals in cytoplasm, plants have developed the competitive mechanism for chelation between heavy metals and low-molecular-weight (LMW) compounds [43]. LMW molecules are either thiol-containing compounds (metallothioneins: MTs, GSH, PCs) or not (e.g., histidine, nicotianamine, etc.).

Heavy metals are intracellularly chelated through the synthesis of amino acids, organic acids, glutathione (GSH), or heavy metal-binding ligands such as metallothioneins (MTs), phytochelatins (PCs) [42].

### 2.3.1. Chelation by Glutathione

Recent research studies provided experimental evidence that a strong antioxidant system, including high glutathione (GSH) levels, is present in different hyper-accumulators and that it is either needed or at least a beneficial trait in metal tolerance [62]. Glutathione (GSH), a nonenzymatic antioxidant, is a low molecular weight thiol implicated in a wide range of metabolic processes and constitutes an important plant defense system against environmental stresses, including HMs [42, 63]. Seth et al. [43] reported that GSH plays a crucial role, as it is not only important in metal chelation, but also in antioxidative defence and redox signalling, as well as in plant growth and development. Glutathione is a precursor for PCs synthesis, is also involved in the detoxification of toxic oxygen species [64], and is generated in response to pesticides [65]. GSH removes metals directly through chelation which process is catalyzed by the glutathione S-transferase [66]. Hossain et al. [42] suggested that GSH protects proteins against denaturation caused by the oxidation of protein thiol groups under stress and plays an indirect role in protecting membranes by maintaining  $\alpha$ -tocopherol and zeaxanthin in the reduced state. Ahner et al. [67] reported that GSH has other functions, including the formation of phytochelatins, which have an affinity for heavy metals and are transported as complexes into the vacuole, thus allowing plants to have some level of resistance to heavy metals. Transgenic plants expressing glutathione (GSH) offer great promise for enhancing the efficiency of Cd phytoextraction from polluted soils and wastewater. These plants may also show increased tolerance to, and accumulation of, other heavy metals, because PCs are thought to play a role in tolerance of a range of heavy metals, especially nonessential heavy metals such as mercury and lead [1].

Torricelli et al. [68] analysed the GSH levels in wild type and chromium-tolerant strains of *Scenedesmus acutus*. The authors found that tolerant strain



showed higher levels of reduced glutathione when the cells were exposed to Cd<sup>2+</sup>.

In the green seaweeds there was an increase in the concentration of GSH with time of exposure to Cd (approximately two-fold higher in *Ulva lactuca* than in *Codium fragile*) when compared to controls [69]. However, in the red seaweed *Gracilaria gracilis* the concentration of GSH did not change. This is probably a consequence of lower intracellular accumulation of Cd by red than brown seaweeds, as previously reported for *Gracilaria tenuistipitata* and *Sargassum thunbergii* [70]. A reduction in the concentration of GSH with increased production of PCs was also evident when *Fucus serratus* was exposed to Zn, but the decrease was apparent after only 4 days [69].

Agrawal et al. [71] analysed the effect of different Hg treatments on glutathione content in a green algae *Chlorogonium eleongatum* (Dang) and they found that Mercury treatments increased the concentration of total glutathione, including both oxidized (GSSG) and reduced (GSH) Glutathione. The two brown seaweeds *Fucus serratus* and *Fucus vesiculosus*, living in an environment with high concentrations of metals, maintained high concentrations of GSH despite synthesis of PCs, although the proportions of GSH to PCs differed between the two species; *Fucus vesiculosus* had a higher proportion of GSH [69]. Wu and Lee [72] showed that total GSH level did not change in *Ulva fasciata* exposed to increasing concentrations of copper, but GSH level increased in *Ulva lactuca* exposed to cadmium excess [73]. Thus, ulvophytes showed different antioxidant responses to heavy metals and, until now, *Ulva compressa* is the only ulvophyte showing GSH synthesis in response to copper excess [74].

### 2.3.2. Chelation by Phytochelatins

Phytochelatins (PCs), small sulphur-rich oligopeptides of the general structure (Glu-Cys)*n*-Gly, *n* = 2–11 and synthesized from reduced glutathione (GSH), are involved in homeostasis and detoxification of metals in the cells of higher plants, eukaryotic microalgae, some fungi. PCs are synthesized from GSH; the metal binds to the constitutively expressed enzyme  $\gamma$  - glutamylcysteinyl dipeptidyl transpeptidase (PC synthase), thereby activating it to catalyze the conversion of GSH to phytochelatin [75].

Algal species can respond to heavy metal exposure by synthesizing metal binding proteins known as phytochelatins [76]. Gekeler et al. [77] demonstrated that algae sequester heavy metals by an identical mechanism as higher plants, namely via complexation to phytochelatins. The two standard characteristics attributed to the PCs are that (1) PC synthesis can be stimulated

in cells exposed to various metal ions such as Cd, Cu, Hg, Ni, Zn, Pb and Ag and (2) the formed PCs are capable of binding to multiple types of metals and metalloids [78, 79]. After the activation of PC synthase by the HM ions and HM chelation by the PCs synthesized, the HM ion complex is transported to the vacuole and stabilized there by forming a complex with sulfides or organic acid [80].

Some seaweeds show a high capacity for accumulation of heavy metals as results of tolerance mechanisms and many algae synthesize phytochelatin (PCs) that can form complexes with heavy metals and translocate them into vacuoles [44]. Pawlik-Skowrońska et al. [69] showed that seaweeds differ in their capacity to produce PCs, even when growing under the same environmental conditions and levels of pollution and the production of PC depends on factors such as morphology, biochemical composition (e.g., polysaccharides), intracellular metal accumulation and the status of the precursor for PC production.

Mellado et al. [74] reported that copper induced the synthesis of ascorbate, glutathione and PCs in *Ulva compressa* suggesting that these compounds are involved in copper tolerance. The total concentration of PCs measured in *Fucus* spp. and *S. chordalis* were positively correlated with the levels of metal contamination at the sites sampled and with the total concentrations of metals in the seaweeds [69]. Yadav [81] reported that PCs are synthesized inductively by exposure to not only Cd, but also by other heavy metals such as Hg, Cu, Zn, Pb and Ni. During the exposure of plants to such metals, PCs are synthesized from GSH by phytochelatin synthase (PCS) activity. Thus, marine macroalgae differ in their abilities to synthesize PCs in response to heavy metals and, in particular, *Ulva compressa* is the only ulvophyte showing synthesis of PCs in response to copper excess.

PCs ensure the homeostasis of Cu and Zn by transferring them to the apoenzymes in the necessary amount. The remaining amount of these metals are transferred to the vacuoles [75]. Recently, Roncarati et al. [82] studied the intra-specific responses to Cu-stress in two strains of the brown alga *Ectocarpus siliculosus* (Es524 and LIA), and they found that the higher intracellular concentrations of Cu, lower production of PCs, and lower expression of enzymes involved in GSH-PCs synthesis may be contributing to an induced oxidative stress condition in LIA, which explains, at least in part, the observed sensitivity of LIA to Cu. In Es524, there was an increase in the transcripts of  $\gamma$ -GCS, GS and PCS, particularly under high Cu exposure. Ahner et al. [67] reported that the 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. Increased production of PCs was also evident in the red seaweed, *G. gracilis*, upon exposure to Cd. Given that concentrations of PCs vary with intracellular concentrations of metals [83, 84], the data from the field and experimental studies suggest that the concentrations of PCs in seaweeds are mechanistically linked to uptake of metals and that they reflect the bioavailability of metals.

### 2.3.3. Chelation by Metallothioneins

Metallothioneins (MTs) are low molecular mass cysteine-rich proteins that can bind heavy metals such as Cd, Zn and Cu in thiol groups [85, 86] and can participate in the homeostasis of intracellular metal ions [87]. Since their discovery as Cd-binding proteins present in horse kidney, MT proteins and genes have been found throughout the animal and plant kingdoms as well as in the prokaryote *Synechococcus* and seaweeds [50, 88]. Ahn et al. [89] reported that plant MTs are thought to be primarily involved in cellular ion homeostasis. While it is likely that plant PCs and MTs both participate in heavy metal detoxification, their distinctive roles have not been clearly demonstrated. In the absence of MTs, or another ligand, free copper ions would precipitate a cascade of oxidative damage and disrupt the controlled senescence program [50]. Metal coordination takes place through the large number of cysteine sulfurs present in the protein forming two metal-binding domains in mammalian, crustacean, plant, and algal MTs [88-92]. Castiglione et al. [93] reported that besides detoxification, MTs may also take part in the regulation of gene expression and cell metabolism, by donating/accepting metal ions (e.g., Zn) to/from metal-dependent DNA-binding proteins or metalloenzymes [93]. The peptide is synthesized by some seaweed, which chelates metals and stores them in compartments within cell or segregates them from surrounding environment, hence preventing free heavy metal circulation inside the cytosol [94]. Since other stresses, like heat shock and aluminium, also induce this type of expression, it was suggested that these MTs might express as part of a general stress response [50]. There is, however, some evidence to suggest that MTs are involved in copper homeostasis and detoxification [95]. The biosynthesis of MTs is regulated at the transcriptional level and is induced by several factors, including hormones, cytotoxic agents, and HMs, such as Cd, Zn, Hg, Cu, Au, Ag, Co, Ni, and Bi [96, 97].

Morris et al. [88] identified and characterized the gene for metallothionein in brown seaweed *Fucus vesiculosus*. Recombinant metallothionein from

brown seaweed *Fucus vesiculosus* (rfMT)1 has been reported to remediate arsenic [98].

#### **2.3.4. Chelation by Amino Acids, phytate and Organic Acids**

Besides the thiol-containing compounds (metallothioneins: MTs, GSH, PCs), other classes of metal-chelating agent have also been used for metal homeostasis, detoxification and tolerance (e.g., citrate, proline, malate, oxalate, nicotianamine (NA), histidine (His), phytate etc.) [99]. Kim et al. [100] found that transgenic *Arabidopsis* and tobacco plants that constitutively overexpress the barley nicotianamine synthase gen increased nicotianamine biosynthesis and conferred enhanced tolerance of high levels of metals particularly nickel. However, Hanikenne et al. [101] reported that phytosiderophore or his precursor nicotianamine are not found in algae and this function may thus have appeared after the emergence of land plants.

Amino acids and their derivatives, such as histidines, glycine, betaine, proline, arginine, glutamate and cysteines, in isolation and/or in coordination with thiol compounds contribute to metal chelation in plants [45, 62, 102]. Sharma and Dietz [62] reported that upon exposure to metals, plants often synthesize a set of diverse metabolites that accumulate to concentrations in the millimolar range, particularly specific amino acids, such as proline and histidine, peptides such as glutathione and the amines spermine, spermidine, putrescine, nicotianamine, and mugineic acids [62]. Various peptides consisting of metal-binding amino acids (mainly histidine and cysteine residues) have been studied for enhanced heavy metal accumulation by bacteria [103, 104]. Histidine (His) has a high capacity to chelate heavy metals. The histidyl dipeptide *carnosine* (b-alanyl-l-histidine) with antioxidant activity thought to be associated with its ability to chelate transition metals was also characterized in red seaweed *Ancanthophora delilei* [105].

Organic acids such as malate, oxalate, aconitate, malonate, tartrate and citrate have been evidenced to contribute to metal chelation [45, 62, 102, 106]. Wuana et al. [107] suggested that citrate appeared to offer greater potentials as chelating agents for heavy metals. Citric acid has been considered a major ligand at low Cd concentrations [108]. Jauregui-Zuniga et al. [109] showed that calcium oxalate could play an important role in heavy metal detoxification. Previously Mathys [110] proposed that malate chelated Zn in the cytosol and the complex was moved into the vacuole, where the uniformly abundant oxalate chelated the Zn, to free malate for return to the cytosol. Another ligands that can bind metals such as Zn and Al are phytates [111].

Phytate are potential ligands for heavy metals and are found to play a role in tolerance and detoxification [42].

### **3. ROLE OF SEAWEED IN BIOMONITORING OF WATER POLLUTION**

Biomonitoring (Biological monitoring) is the specific application of biological response for the evaluation of environmental change for using this information in quality control program. Recently, there has been a growing interest in using algae for biomonitoring eutrophication, organic and inorganic pollutants, despite some problems associated with seasonal variations, temperature and salinity conditions and intrinsic factors such as age and growth rate [112-117]. Algae are an ecologically important group in most aquatic ecosystems and have been an important component of biological monitoring programs [118].

Chaudhuri et al. [119] reported that macroalgal species could be good biomonitors of contaminants that tend to reside in the dissolved phases (like heavy metals) compared to those contaminants that are lipophilic (like organochlorines). These lipophilic contaminants will not be readily taken up by macroalgal species, due to their low lipid content.

As an alternative to the direct determination of heavy metals in seawater, these can be assayed in a suitable biomonitor, namely a marine macroalgae, and knowing the corresponding concentration factors, the mean metal contents in seawater can be estimated [112]. Seaweeds are used as bioindicators because of their distribution, size, longevity, presence at pollution sites, ability to accumulate metals to a satisfactory degree and ease of identification [120-122]. Wan Maznah [118] suggested that algae are ideally suited for water quality assessment because they have rapid reproduction rates and very short life cycles, making them valuable indicators of short-term impacts.

The seaweeds species can directly reflect the water quality assessment because they are sensitive to some pollutants, and algal metabolism is sensitive to the variation of environmental and natural disturbances [118]. The seaweeds are used in biomonitoring because they are easily cultured in the laboratory and sampling is easy, inexpensive and creates minimal impact on resident biota; relatively standard methods exist for the evaluation of functional and non-taxonomic structural characteristics of algal communities [123-127].

The brown algae *Cystoseira* sp., and the green algae *Ulva* sp. and *Enteromorpha* sp. have high potential as cosmopolitan biomonitors for trace metals in the Aegean Sea [128]. Previously, Leal et al. [112] suggested that the marine benthic macroalgae *Enteromorpha* spp. and *Porphyra* spp. can be used as biomonitors of the seawater contents of Cd, Cu, Hg and Pb. In recent years, several species of the green algae *Enteromorpha* and/or *Cladophora* have been utilized to measure heavy metal levels in many parts of the world [129]. The high uptake of metals in green algae (*Ulva lactuca* and *Enteromorpha intestinalis*) and brown algae (*Padina gymnospora* and *Dictyota bartayresiana*) suggested that these algae may be used as potential biomonitors for heavy metal pollution [130]. Wong et al. [131] used *Enteromorpha crinita* as a biomonitor in the Hong Kong waters, and Say et al. [132] advocated the use of *Enteromorpha* species as biomonitors in temperate coastal waters.

#### 4. BIOREMEDIATION BY SEAWEEDS

Bioremediation is the use of seaweeds or other organisms to reduce the concentrations or toxic effects of contaminants in the aquatic ecosystems. Phytoremediation is defined as a process of decontaminating soil and aquatic systems by using plants, fungi or algae to remove or degrade organic and inorganic pollutants [1]. Zhou et al. [133] defined the bioremediation as a scientific technique for assessing environment including human exposures to natural and synthetic chemicals, based on sampling and analysis of an individual organism's tissues and fluids. This technique takes advantage of the knowledge that chemicals that have entered the organisms leave markers reflecting this exposure.

However, phycoremediation is defined as a process of decontaminating soil or aquatic systems by using microalgae or seaweeds. Such a process has been used to clean up heavy metals, toxic aromatic pollutants, acid mine drainage, pesticides and xenobiotics and organic compounds [134].

Recently, the use of aquatic plants especially micro and macro algae has received much attention due to their ability to absorption of metals and taking up toxic elements from the environment or rendering them less harmful [135]. Sivakumar et al. [136] suggested that microalgae are capable of producing lipids and hydrocarbons quickly and their photosynthetic abilities make them a promising candidate for wastewater treatment (bioremediation) and can be used as an alternative energy source (Biodiesel). Worldwide, trees, grasses,

herbs, and associated fungi and microorganisms are being used increasingly for cleaning polluted sites. Phytoremediation is "on the brink of commercialization" [137], and is given a rapidly increasing market potential [138]. The phytoremediation market is still emerging in Europe, while in the US revenues are likely to exceed \$300 million in 2007 [139]. Mudgal et al. [134] suggested that the plant used in the phytoremediation technique must have a considerable capacity of metal absorption, its accumulation and strength to decrease the treatment time. Besides cost-effectiveness, bioremediation is a permanent solution, which may lead to complete mineralization of the pollutant. Furthermore, it is a non-invasive technique, leaving the ecosystem intact. Bioremediation can deal with lower concentration of contaminants where the cleanup by physical or chemical methods would not be feasible [15]. Many green (Table 1), red (Table 2) and brown seaweeds (Table 3) are known to be a good heavy metal hyperaccumulators and can be used in bioremediation of polluted ecosystems.

The seaweeds can eliminate heavy metals by two processes: bioaccumulation and biosorption.

#### **4.1. Bioaccumulation**

Bioaccumulation is a process that allows for binding toxic metals or organic substances inside a cell structure [140]. Bioaccumulation is an active metabolic process driven by energy from a living organism and requires respiration [3, 141]. It has been reported that stronger ligands, as they have been shown to complex metals in non-hyperaccumulators, are in hyperaccumulators used for transient binding during transport to the storage sites [142]. This confirmed that enhanced active metal transport, and not metal complexation, is the key mechanism of hyperaccumulation. The hyperaccumulating plants store metals in the vacuoles because in this organelle only enzymes like phosphatases, lipases, and proteinases [143, 144] are present, which have not been found to be a target of heavy metal toxicity. Suresh and Ravishankar [44], reported that seaweeds proved to be effective in hyperaccumulation of heavy metals as well as degradation of xenobiotics. Many seaweeds are able to accumulate high levels of trace metals (Tables 1, 2, 3), which are sometimes larger than those found in water samples from the same site [41, 145]. Seaweeds are able to accumulate trace metals, reaching concentration values that are thousands of times higher than the corresponding concentrations in sea water [146-148]. Henriques et al. [149] showed that

bioaccumulation as a full remediation process brings great advantages but only if the contaminated water fulfils the criteria of minimal growth medium and exerts no critical toxic effect to cells. Gosavi et al. [150] demonstrated that four genera of macroalgae (*Ulvasp.*, *Enteromorphasp.*, *Chaetomorpha* sp. and *Cladophora* sp.) accumulated significant amounts of Fe, Al, Zn, Cd, Cu, As and Pb, noting that cadmium was absorbed better by *Cladophorasp.*, while *Chaetomorpha* sp. and *Enteromorpha* sp. absorbed lead better. Previously, Tukai et al. [151] demonstrated that higher concentrations of as were found in brown seaweeds when compared with the red and green species. To study the removal of Hg from water by the living algae, Henriques et al. [149] assessed and explored the bioaccumulation capabilities of three different macroalgae species, *Ulva lactuca* (green), *Gracilaria gracilis* (red) and *Fucus vesiculosus* (brown) and they found that all seaweeds showed huge accumulation capabilities, reaching up 209 µg of Hg per gram of macroalgae (dry weight), which corresponds to 99% of Hg removed from the contaminated seawater. *Ulva lactuca* was the fastest to accumulate Hg. Leitenmaier and Küpper [142] reported that hyperaccumulators have been found for many heavy elements and within many groups of plants and algae, including at least the following: Al, As, Cd, Cu, I, Mn, Ni, Se, Zn [142]. Tonon et al. [152] evaluated the absorption of metals by three species of *Gracilaria*: *Gracilaria tenuistipitata*, *Gracilaria domingensis* and *Gracilaria Birdiae*. The differences between the three species in the concentrations of the various elements, probably is due to physiological, biochemical or genetic differences between the seaweeds or to different acclimatization events that occurred in their environments or microenvironments. These results suggest that some red seaweed is metal bioaccumulating organisms. Pawlik-Skowrońska et al. [69] reported that the two *Fucus* spp., *Fucus serratus* and *Fucus vesiculosus*, accumulated higher total concentrations of metals than either *Ulva intestinalis* or *Solieria chordalis* independently of the level of contamination. This high capacity for accumulation of metals by these seaweeds, and especially *Fucus* spp., when exposed to complex metal mixtures in their natural habitats indicates that they must have effective mechanisms for metal homeostasis and detoxification.

## 4.2. Biosorption

Biosorption is a physiochemical process that occurs naturally in certain biomass which allows it to passively concentrate and bind contaminants onto its cellular structure [153]. Velásquez and Dussan [3] reported that biosorption



is a metabolically passive process, meaning it does not require energy, and the amount of contaminants a sorbent can remove is dependent on kinetic equilibrium and the composition of the sorbents cellular surface.

Biomass from many organisms including fungi and algae has been extensively studied as an alternative adsorbent in removal of heavy metal ions [154, 155]. The mechanism of biosorption is based on a number of metal-binding processes taking place with components of the cell wall [38].

Furthermore, there are other factors affecting the biosorption of metals by seaweed biomass that should be considered, such as cell size and morphology, pH of the external media, cation and anion concentration in the external media, metal speciation, temperature and physiology of the biomass used for the metal [156]. It has been established that metal sequestration is achieved through the following processes: physical sorption, ion exchange, chelation; and ion fixation in inter- and intrafibrillar capillaries and spaces of the structural polysaccharide matrix as a result of the concentration gradient and diffusion through cell walls [157-159]. Previously, Tsezos and Volesky [160] reported that alginates of marine algae usually occur as natural salts of  $K^+$ ,  $Na^+$ ,  $Ca^{2+}$  and/or  $Mg^{2+}$ . These metallic ions can exchange with the counter ions such as  $Co^{2+}$ ,  $Cu^{2+}$ ,  $Cd^{2+}$  and  $Zn^{2+}$ , resulting in the biosorptive uptake of the metals.

It was shown that the brown seaweeds contained the greatest number of acidic functionalities (both total and weak) on the seaweed surface. Since it is thought that carboxyl groups (weak) are primarily responsible for metal sorption, especially in brown seaweeds (Tables 1, 2, 3), it was expected that the brown species would exhibit superior biosorption performance over the other seaweeds [161]. Vijayaraghavan et al. [162] showed that the marine green alga *Ulva reticulata* was found to be an effective biosorbent for the removal of copper, cobalt and nickel from aqueous solutions. Therefore, cell wall composition of green algae provides binding sites such as carboxyl hydroxyl amino and sulphate for metal ions [163-166] and (4) its collection from coastal regions can solve possible eutrophication problem. One of the most common macroalgae used in past studies of heavy metal biosorption from the environment, as well as for the removal of nitrogen and ammonia in fish aquaculture is the green alga *Ulva* [166, 167].

Using green seaweed, Zeroual et al. [168] found that *Ulva lactuca* can successfully used for mercury biosorption. Previously, Kuyucak and Volesky [169] observed that a green alga *Halimeda opuntia* performed equally well in cobalt biosorption along with one of the best-performed brown seaweed *Ascophyllum nodosum* at higher pH conditions.

Murphy et al. [161] studied the biosorption performance of Cu(II) by the dried biomass of the two red seaweeds *Palmaria palmata* and *Polysiphonia lanosa*, and they found that carboxyl and sulphonate functionalities involved in binding Cu(II) in both species. However amino and hydroxyl groups took part in Cu(II) binding in *P. lanosa*.

Alkhalifa et al. [170] suggested that the main mechanisms of heavy metals biosorption by brown algae include some key functional groups such as carboxylic groups, which are generally the most abundant acidic functional group in the brown algae. They constitute the highest percentage of titratable sites (typically greater than 70%) in dried brown algal biomass. The adsorption capacity of the algae is directly related to the presence of these sites on the alginate polymer, which itself comprises a significant component (up to 40% of the dry weight) [170].

**Table 1. Uptake and accumulation of metals by some green seaweeds**

Metal	Species	References
As	<i>Codium cuneatum</i>	[171]
	<i>Maugeotia genuflexa</i>	[172]
	<i>Rhizoclonium tortuosum</i>	[69]
	<i>Ulothrix cylindricum</i>	[173]
B	<i>Caulerpa racemosa</i>	[174]
Ba	<i>Codium cuneatum</i>	[171]
Cd	<i>Enteromorpha sp.</i>	[128]
	<i>Cladophora fascicularis</i>	[175]
	<i>Codium tomentosum</i>	[176]
Co	<i>Enteromorpha intestinalis</i>	[176]
	<i>Ulva lactuca</i>	[176]
Cr	<i>Enteromorpha sp.</i>	[128]
	<i>Ulva sp.</i>	[177]
Cu	<i>Codium tomentosum</i>	[176]
	<i>Enteromorpha sp.</i>	[112]
	<i>Rhizoclonium tortuosum</i>	[69]
	<i>Ulva lactuca</i>	[121]
	<i>Ulva sp.</i>	[128, 130]
Fe	<i>Codium cuneatum</i>	[171]
	<i>Enteromorpha sp.</i>	[128]
	<i>Ulva lactuca</i>	[130, 178]
Hg	<i>Enteromorpha sp.</i>	[112, 128]
	<i>Ulva lactuca</i>	[149]
Mn	<i>Ulva lactuca</i>	[176]

**Table 1. (Continued)**

Metal	Species	References
Ni	<i>Ulva lactuca</i>	[176]
	<i>Enteromorpha intestinalis</i>	[176]
Pb	<i>Cladophora fascicularis</i>	[175]
	<i>Enteromorpha sp.</i>	[128, 130]
	<i>Rhizoclonium tortuosum</i>	[69]
Sr	<i>Codium cuneatum</i>	[171]
Zn	<i>Enteromorpha sp.</i>	[128]
	<i>Rhizoclonium tortuosum</i>	[69]
	<i>Ulva lacuca</i>	[121, 130]
	<i>Ulva reticulata,</i>	[179]

**Table 2. Uptake and accumulation of metals by some red seaweeds**

Metal	Species	References
Cd	<i>Gelidium floridanum</i>	[180]
	<i>Gracillaria compressa</i>	[176]
	<i>Kappaphycus alvarezii</i>	[181]
	<i>Porphyra spp.</i>	[112]
	<i>Pterocladia capillacea</i>	[176]
Co	<i>Gracillaria compressa</i>	[176]
	<i>Jania rubens</i>	[176]
	<i>Kappaphycus alvarezii</i>	[181]
	<i>Pachymeniopsis sp</i>	[182]
	<i>Polysiphonia lanosa</i>	[183]
Cu	<i>Gracillaria compressa</i>	[176]
	<i>Porphyra spp.</i>	[176]
	<i>Solieria chordalis</i>	[69]
Fe	<i>Gracilaria pachidermatica</i>	[171]
	<i>Laurencia papilosa</i>	[171]
	<i>Pterocladia capillacea</i>	[176]
Hg	<i>Gracilaria gracilis</i>	[149]
	<i>Porphyra spp</i>	[112]
Ni	<i>Gracillaria compressa</i>	[176]
	<i>Gracillaria verrucosa</i>	[176]
Pb	<i>Gracilaria pachidermatica</i>	[171]
	<i>Gelidium floridanum</i>	[180]
	<i>Porphyra spp</i>	[112]
Se	<i>Gracilaria edulis</i>	[183]
Sr	<i>Laurencia papilosa</i>	[171]
Zn	<i>Pterocladia capillacea</i>	[176]

**Table 3. Uptake and accumulation of metals by some brown seaweeds**

Metal	Species	Reference
As	<i>Fucus serratus</i>	[69]
Au	<i>Ascophyllum nodosum</i>	[185]
	<i>Chondrus crispus</i>	[185]
	<i>Palmaria palmata</i>	[185]
	<i>Palmaria tevera</i>	[185]
	<i>Rhodymenia palmata</i>	[186]
	<i>Sargassum natans</i>	[185]
Ba	<i>Padina durvillaei</i>	[171]
	<i>Sargassum sinicola</i>	[171]
Cd	<i>Ascophyllum nodosum</i>	[187]
	<i>Cystoseira sp.</i>	[128]
	<i>Fucus vesiculosus</i>	[187]
	<i>Padina gymnospora</i>	[130]
	<i>Sargassum natans</i>	[187]
	<i>Turbinaria conoides</i>	[141]
Co	<i>Ascophyllum nodosum</i>	[169]
Cr	<i>Cystoseira sp.</i>	[128]
	<i>Dictyota bartayresiana</i>	[130]
	<i>Fucus vesiculosus</i>	[183, 188]
	<i>Sargassum sp.</i>	[141]
	<i>Turbinaria conoides</i>	[141]
Cu	<i>Fucus serratus</i>	[189]
	<i>Padina pavonica</i>	[121]
	<i>Rhizoclonium tortuosum</i>	[69]
	<i>Sargassum boveanum</i>	[170]
	<i>Sargassum filipendula</i>	[190]
	<i>Sargassum fluitans</i>	[190]
	<i>Turbinaria conoides</i>	[141]
Fe	<i>Fucus vesiculosus</i>	[188]
	<i>Cystoseira sp.</i>	[128]
	<i>Padina durvillaei</i>	[171]
	<i>Sargassum fluitans</i>	[191]
	<i>Sargassum sinicola</i>	[171]
Hg	<i>Cystoseira sp.</i>	[128]
	<i>Fucus vesiculosus</i>	[149]
Mn	<i>Padina gymnospora</i>	[130]
Ni	<i>Ascophyllum nodosum</i>	[192]
	<i>Fucus vesiculosus</i>	[188, 192]
	<i>Padina gymnospora</i>	[130]
	<i>Sargassum fluitans</i>	[192]
	<i>Sargassum natans</i>	[192]

**Table 3. (Continued)**

Metal	Species	References
Pb	<i>Ascophyllum nodosum</i>	[192]
	<i>Cystoseira sp.</i>	[128]
	<i>Fucus vesiculosus</i>	[192]
	<i>Sargassum natans</i>	[192]
	<i>Sargassum vulgare</i>	[192]
	<i>Turbinaria conoides</i>	[141]
Sb	<i>Turbinaria conoides</i>	[193]
	<i>Sargassum sp</i>	[193]
Sr	<i>Padina durvillaei</i>	[171]
	<i>Sargassum sinicola</i>	[171]
Zn	<i>Cystoseira sp.</i>	[128]
	<i>Fucus vesiculosus</i>	[194]
	<i>Laminaria japonica</i>	[194]
	<i>Padina pavonica</i>	[121]
	<i>Sargassum angustifolium</i>	[170]
	<i>Sargassum fluitans</i>	[194]
	<i>Sargassum latifolium</i>	[170]

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