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The role of algae in phytoremediation of heavy metals: A review

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Abstract

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. The accumulation of these pollutants is due to intensive anthropogenic activity. These problems of pollution can be partially solved by the application of phytoremediation technologies using algae or aquatic plants to remove pollutants from the environment. In this review, we will present different techniques used in bioremediation and the ability of some micro and macroalgae species to absorb the organic and inorganic pollutants. Some algae are characterized by fast growth rate which is an advantage for phytoremediation, but there are also some factors which may limit the efficacy of phytoremediation of contaminated sites that will also be reviewed.

Key words: Algae, Phytoremediation, Pollution, Heavy metals

1. Introduction

Organic pollutants and heavy metals are considered to be a serious environmental problem for human health. The contamination of soils and aquatic systems by toxic metals and organic pollutants has recently increased due to anthropogenic activity.

Phytoremediation has emerged as the most desirable technology which uses plants for removal of environmental pollutants or detoxification to make them harmless [1]. Many living organisms can accumulate certain toxicants to body concentrations much higher than present in their environments [1-4]. Thus, the use of plants for the decontamination of heavy metals has attracted growing attention because of several problems associated with pollutant removal using conventional methods. Bioremediation strategies have been proposed as an attractive alternative owing to their low cost and high efficiency [5].

Recently, there has been a growing interest in using algae for biomonitoring eutrophication, organic and inorganic pollutants. By using the chlorophyll formation of the algae, for example, it was possible to estimate spectrophotometrically the total nitrogen content in water collected from aquatic systems [6] giving us an idea on eutrophication levels [7].

The plant used in the phytoremediation technique must have a considerable capacity of metal absorption, its accumulation and reducing the time of decontamination of an ecosystem [8].

Plants are known to be able to accumulate many heavy metals [9]. Heavy metal tolerance in plants may be conferred by their immobilization in the cell wall [9,10], or by their compartmentalization in vacuoles [11]. Some algae show a high capacity for accumulation of heavy metals as results of tolerance mechanisms and many algae synthesize phytochelatins and metallothioneins that can form complexes with heavy metals and translocate them into vacuoles [12].

Another advantage of the use of the algae in phytoremediation is the high biomass production by these species leading to high absorption and accumulation of heavy metals. In potato plants, we found the positive correlation between organ biomass and absorption and accumulation of heavy metals [9]. Phytoremediation is potentially applicable to a variety of contaminants, including some of the most significant ones, such as petroleum hydrocarbons, chlorinated solvents, metals, radionuclides, nutrients, pentachlorophenol (PCP), and polycyclic aromatic hydrocarbons (PAHs). Phytoremediation technologies are becoming recognized as costeffective methods for remediating sites contaminated with toxic metals at a fraction of the cost of conventional technologies, such as soil replacement, solidification and washing strategies [13,14]

In the present review we present an overview of the origin of organic and inorganic pollutants in aquatic ecosystems, and different mechanisms used by algae and aquatic higher plants to accumulate theses pollutants to minimize their impact on environment.

2. Origin of heavy metals in aquatic ecosystems

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 [15]. 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 [16]. Studying the bioavailability and origin of heavy metals from the Nador lagoon sediments, González *et al.* [17] 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 [18].

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 [19]. 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 [20]. Wang *et al.* [21] 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 [21].

Okafor and Opuene, [22], Mohiuddin *et al.* [23] reported that trace elements may be immobilised within the stream sediments and thus could be involved in absorption, coprecipitation, and complex formation. Sometimes they are co-adsorbed with other elements as oxides, hydroxides of Fe, Mn, or may occur in particulate form [24, 25, 44].

However, 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 [26-28].

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 [29].

3. Phytoremediation of heavy metals by algae

Heavy metal removal mechanisms include sedimentation, flocculation, absorptionand cations and anion exchange, complexation, precipitation, oxidation/reduction, microbiological activity and uptake. Microalgae remove heavy metals directly from polluted water by two major mechanisms; the first is a metabolism dependent uptake into their cells at low concentrations, the second is biosorption which is a non-active adsorption process [30].

Phytoremediation is defined as a process of decontaminating soil and aquatic systems by using plants, fungi or algae to absorb heavy metals. 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 [31].

The algae have many features that make them ideal candidates for the selective removal and concentration of heavy metals (Table 1), which include high tolerance to heavy metals, ability to grow both autotrophically and heterotrophically, large surface area/volume ratios, phototaxy, phytochelatin expression and potential for genetic manipulation [32]. Macroalgae have been used extensively to measure heavy metal pollution and marine environments throughout the world. 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 [33].

Table 1: Uptake and accumulation of metals by some algal species

Species	Metal	References
Ascophyllum nodosum	Gold (Au)	Kuyucak and Volesky [67]
	Cobalt (Co)	Kuyukak and Volesky [68]
	Nickel (Ni)	Holan and Volesky [69]
	Lead (Pb)	Holan and Volesky [69]
Caulerpa racemosa	Boron (B)	Bursali *et al.* [43]
Daphnia magna	Arsenic (As)	Irgolic *et al.* [70]
Fucus vesiculosus	Zinc (Zn)	Fourest and Volesky [71]
	Nickel (Ni)	Holan and Volesky [69]
Laminaria japonica	Zinc (Zn)	Fourest and Volesky [71]
Micrasterias denticulata	Cadmium (Cd)	Volland *et al.* [72]
Phormedium bohner,	Cromium (Cr)	Dwivedi *et al.* [73]
Platymonas subcordiformis	Strontium (Sr)	Mei *et al.* [38]
Sargassum filipendula	Copper (Cu)	Davis *et al.* [74]
Sargassum fluitans	Copper (Cu)	Davis *et al.* [74]
	Iron (Fe)	Figueira *et al.* [75]
	Zinc (Zn)	Fourest and Volesky [71]
	Nickel (Ni)	Holan and Volesky [69]
Sargassum natans	Lead (Pb)	Holan and Volesky [69]
Sargassum vulgare	Lead (Pb)	Holan and Volesky [69]
Spirogyra hyalina	Cadmium (Cd), Mercury (Hg), Lead (Pb), Arsenic (As) and Cobalt (Co)	Kumar and Oommen [76]
Tetraselmis chuil	Arsenic (As)	Irgolic *et al.* [70]

The ability of macroalgae to accumulate metals within their tissues has led to their widespread use as biomonitors of metal availability in marine systems [34,35].

Therefore *Chlorophyta* and *Cyanophyta* are hyper-absorbents and hyper-accumulators for Arsenic and Boron [36], absorbing and accumulating these elements from their environment into their bodies. These algae can be hyper-phytoremediators and their presence in water reduces water Arsenic and Boron pollutant [36].

The brown algae (*Phaeophyta*) are particularly efficient accumulators of metals due to high levels of sulfated polysaccharides and alginates within their cell walls for which metals show a strong affinity [10]. Nielsen *et al.* [37] suggested that brown algae such as *Focus spp.* often dominate the vegetation of heavy metal-contaminated habitats (Figure 1).

Figure 1. Brown algae “*Fucus serratus*”

Mei *et al.* [38] suggested that *Platymonas subcordiformis*, a marine green microalgae (Figure 2), had a very strontium uptake capacity, al-though high concentrations of strontium cause oxidative damage, as evidenced by the increase in lipid peroxidation in the algal cell samples and the decrease in growth rate and chlorophyll contents.

Figure 2. *Platymonas subcordiformis* a marine green microalgae

Studying the capacity of three cultivated aquatic macrophytes, *Lemna minor*, *Elodea canadensis* and *Cabomba aquatica*, in removing copper, Olette *et al.* [39] found that duckweed (*Lemna minor)* showed better potential for removing Cu than did *E. canadensis* and *C.aquatica.*

Some algal species may convert mercuric or phenylmercuric ions into metallic mercury which is then volatilized out of the cell and from the solution [40,41]. The blue green alga *Phormidium* (Figure 3A) successfully can hyperaccumulate heavy metals like Cd, Zn, Pb, Ni and Cu [42]. *Caulerpa racemosa* var. *cylindracea* (Figure 3B) as a low cost biomaterial could be used for the removal of boron species from aqueous solution [43].

Dunaliella salina, a green microalgae, have high tendency for zinc accumulation followed by copper and cobalt, the lowest tendency was for cadmium, this may be due to the importance of zinc as hydrogen transferring in photosynthesis [44].

The principal mechanism of metallic cation sequestration involves the formation of complexes between a metal ion and functional groups on the surface or inside the porous structure of the biological material. The carboxyl groups of alginate play a major role in the complexation. Different species of algae and the algae of the same species may have different adsorption capacity [45].

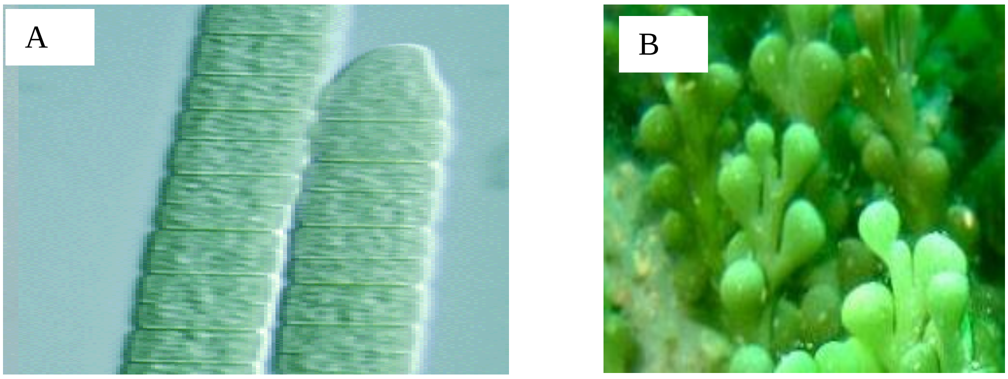

Figure 3. blue green alga *Phormidium* (A) and *Caulerpa racemosa* var. *cylindracea* (B)

4. Heavy Metal Accumulation and tolerance Mechanism's in algae

Organisms respond to heavy metal stress using different defense systems (Table 2), such as exclusion, compartmentalization, making complexes and the synthesis of binding proteins such as metallothioneins (MTs) or phytochelatins (PCs) and translocate them into vacuoles [5]. 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 [46-52].

Studying the absorption of heavy metals in higher plants, we found the greater accumulation of Al and B in our experiment may be related to the high concentrations of pectins in the cell wall. This would provide the plants a strong tolerance to heavy metals and, therefore, greater phytoextraction [9]. 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 [53].

Two marine algae, *Thalassiosira weissflogii* and *Thalassiosira pseudonana*, produce phytochelatins in great amounts due to the higher activity of phytochelatin synthase, which has greater affinity for the glutathione substrate or metal ions [54]. Other algae, such as *Spiruline* (Figure 4A) and *Chlamydomonas* (Figure 4B), also were studied for their ability to accumulate heavy metals like Zn, Pb and Cu [55].

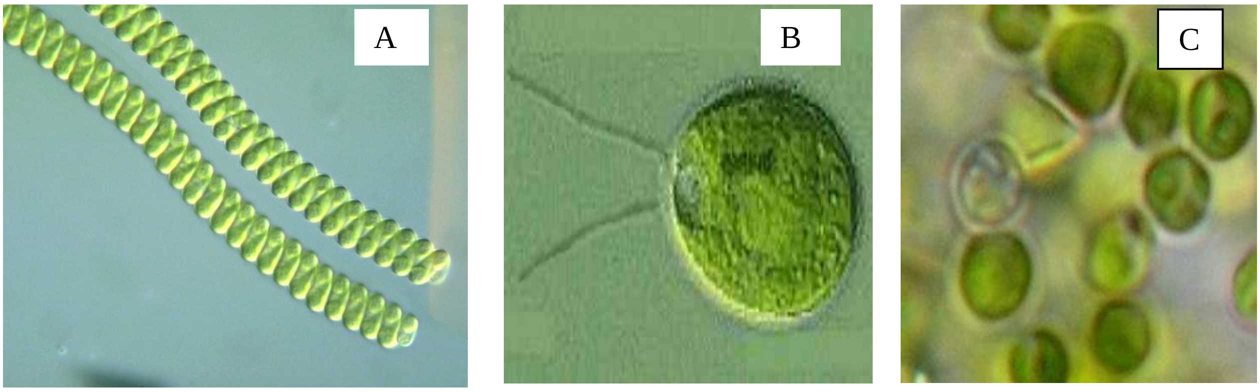

Figure 4. *Spiruline* (A) and *Chlamydomonas* (B) and *Chlorella vulgaris* microalgae (C).

Previously, Mehta and Gaur [56], suggested that proline accumulation in green algae *Chlorella vulgaris* (Figure 4C) 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. The Ni-hyperaccumulation plant species (Brassicaceae) has been demonstrated to be specifically linked to the ability for free histidine production [57,58].

Table 2: Detofixication mechanisms of heavy metals by algae

Metal	**Detoxification mechanisms**	**References**
Cd, Cu, Ag, Hg, Zn, and Pb	Metallothioneins (MTs) and Phytochelatins (PCs).	Clemens *et al.* [77], Vatamaniuk *et al.* [78], Ha *et al.* [79], Volland *et al.* [72]
Ni	Histidine	Krämer *et al.* [57]
Pb, Cu, Cd, Zn, Ca	Cell wall components (Alginates and guluronic acid, sulfated polysaccharides and alginates)	Sekabira *et al.* [53], Davis *et al.* [74], Gupta *et al.* [80].

5. Role of genetic engineering in developing plants for phytoremediation

A number of bacteria and algae have been genetically engineered to remove a specific heavy metal from contaminated water by overexpressing a heavy metal binding protein, such as metallothionein, along with a specific metal transport system. This was first done with an Hg^{++} transport system [59,60].

The introduction and/or overexpression of metal binding proteins have been widely exploited to increase the metal binding capacity, tolerance or accumulation of bacteria and plants. Modification in the biosynthesis of phytochelatins (PCs) in plants has recently been accomplished to enhance the metal accumulation whereas in bacteria, various peptides consisting of metal-binding amino acids (mainly histidine and cysteine residues) have been studied for enhanced heavy metal accumulation by bacteria [5,60].

An important pathway by which plants detoxify heavy metals is through sequestration with heavy-metal-binding peptides called phytochelatins or their precursor, glutathione (GSH). The Indian mustard (*Brassica juncea*) transgenic plants overexpressing *gshII* (gene encoding glutathione synthetase) accumulated significantly more Cd than the wild type, and Cd accumulation and tolerance were correlated with the *gshII* expression level [61]. Transgenic plants expressing 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. Another enzyme responsible for catalyzing the biosynthesis of phytochelatins from GSH is phytochelatin synthase. This mechanism protects vascular plants and some algae, fungi and invertebrate from toxic effects of non-essential heavy metals such as As, Cd or Hg [62], and the heterogeneous overexpression of phytochelatins gene (PCS) in *Nicotiana glauca* R. Graham (shrub tobacco) elevated the production of phytochelatins (PCs) and higher accumulation of double of lead (Pb) than in wildtype [63].

Bang *et al.* [64] reported that expression of the thiosulfate reductase gene from *Salmonella typhimurium* in *Escherichia coli* led to increase of the efficiency of removal heavy metals from solution and accumulation of cadmium up to 150 mM in 98% cells.

Guo *et al.* [65] demonstrated that simultaneous expression of *Allium sativum* phytochelatins synthase (AsPCS1) and yeast cadmium factor 1 (YCF1) in *Arabidopsis* led to elevate the tolerance to Cd and As and have higher amounts accumulation of these metals than in the corresponding single-gene transgenic lines and wildtype. Such a stacking of modified genes involved in chelation of toxic metals by thiols and vacuolar compartmentalization represents a highly promising new tool for use in phytoremediation efforts.

Nicotianamine (NA), a chelator of metals, is ubiquitously present in higher plants. Expressing a nicotianamine synthase cDNA (TcNAS1) isolated from the polymetallic hyperaccumulator *Thlaspi caerulescens* in *Arabidopsis thaliana*, Pianelli *et al.* [66] found that transgenic plants expressing TcNAS1 over-accumulate 100-fold more NA than wild type plants and increased nickel tolerance in these transgenic plants.

Conclusions

The accumulation of heavy metals by micro and macroalgae provides an advantage for phytoremediation over other methods which are more costly and not environmental friendly. Therefore, there is a need to improve the possibilities of accumulation of heavy metals in algae, using the genetic engineering to develop transgenic species overexpressing phytochelatins and metallothioneins that can form complexes with heavy metals and translocate them into vacuoles to maximize phytoaccumulation and the removal of toxic elements from the aquatic ecosystems.

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