Review Article

Microalgae as an Indispensable Tool against Heavy Metals Toxicity to Plants: A Review

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Accepted on: 25-12-2014; **Finalized on:** 28-02-2015.

ABSTRACT

The presence of excess amount of heavy metals in different water bodies is a matter of great concern. It is mandatory to know the presence of heavy metals and control them up to a certain level to avoid adverse effects. Here, cyanobacteria work as an alternative to conventional methods to culminate the problem to a greater extent. The conventional methods which employed earlier were expensive means of removing heavy metals whereas microalgae offers the best biological approach to treat waste water as they have the potential to increase O_2 content of waters by photosynthesis and sorption of heavy metals contaminated waters by reducing the cellular antioxidant activity. In this review article, study has been done on various well known methods for removing heavy metals impurities, those were reported by various researchers as well as using algae as an alternative mean i.e. phytoremediation. The studies reported by different researchers regarding different heavy metals uptake by algae, its accumulation in aquatic plants and phytoremediation. Microalgae have played an important role since years. It possess several inherent properties two of them are their photosynthetic ability and ease to engineer them, which attract researchers to use it in bio industrial applications.

Keywords: Microalgae, Cyanobacteria, Heavy Metals, Bioaccumulation, Phytoremediation, Sorption, Toxicity.

INTRODUCTION

Cyanobacteria are considered as one of the useful organisms which are widely used in food industries and in few biotechnological applications. Although many organisms have been used for the bioindustrial generation of valuable metabolites, the productive potential of cyanobacterial species has remained largely unexplored. Cyanobacteria possess several advantages as organisms for bioindustrial processes, including simple input requirements, tolerance of marginal agricultural environments, rapid genetics and carbon neutral applications. The inferences which would be drawn can be used to select the most desired species for industrial applications by knowing their composition.

Two inherent properties of cyanobacteria make them attractive candidates for use in bioindustrial applications: their photosynthetic capability and their capacity for genetic engineering. The natural diversity and distribution of cyanobacterial species makes them capable of growth in areas which are inhospitable for other agricultural species. Apart from this Cyanobacteria possess several advantages as organisms for bio-industrial processes, including simple input requirements, tolerance of marginal agricultural environments, rapid genetics, and carbon-neutral applications that could be leveraged to address global climate change concerns [1].

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.

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 giving us an idea on eutrophication levels[2].

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.

Metals are elements that occur naturally in rocks in relatively low concentrations. They have useful properties and are important components in our daily life. Metals and metalloids comprise about 75% of the known elements. Only H, B, C, N, P, O, S, halogens, and noble gases are not included in this category. Based on chemical and physical properties (the chemical approach), metals have been classified as light, heavy, and metalloids (semi-metals). The term heavy metals are widely used and refer to metals and metalloids with an atomic density greater than 5 g cm^3. Sometimes the term toxic heavy metal is used to emphasize the impact of these elements on the environment and more specifically on their effect on the biological approach. Since heavy metals exert toxic effects on living Organisms, they are termed toxic heavy metals. Some of the heavy metals, such as copper, nickel, and zinc are at very low concentrations essential for life (also

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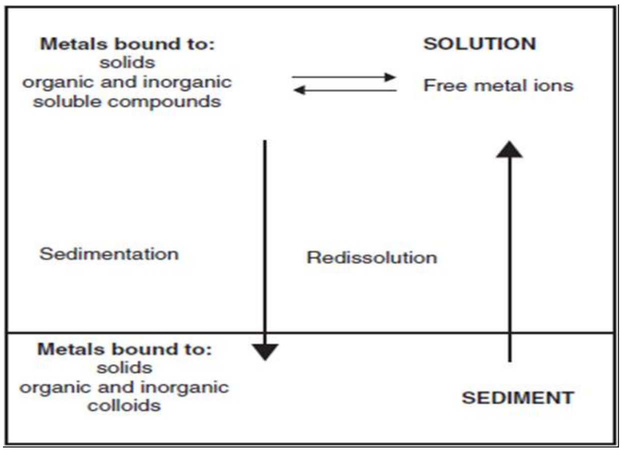
termed microelements or trace elements) because they play important roles in metabolic processes taking place in living cells [3]. However, elevated levels of these metal ions are toxic to most prokaryotic and eukaryotic organisms. Other heavy metals such as cadmium, lead, and mercury are nonessential and are known to cause severe damage in organisms even at very low concentrations. Metals in the environment occur in different chemical forms (metal speciation): as ions dissolved in water as vapours or as salts or minerals in rocks, sand, and soils as shown in figure 1.1. They can also be bound in organic or inorganic molecules or attached to particles in the air [4] . The chemical form of a metal in the environment is constantly changing due to a wide spectrum of dynamic biochemical processes. The latter are influenced by biotic (interactions with living organisms, e.g., microorganisms, plants, and animals) and abiotic factors (e.g., temperature, pH, organic matter, andionic strength) [5]. Metal speciation (chemical forms) is determining metals solubility, mobility, availability, and toxicity. It is generally accepted that for most metals the free ion is the species most toxic to aquatic life [6,7]. Some organic forms such as methyl-mercury are taken up very efficiently by living organisms. It is more toxic than other mercury species[8]. A wide range of anthropogenic activities contribute to the discharge of heavy metals to the environment for example, intensive agriculture, metallurgy, energy production, and microelectronic and sewage sludge. Heavy metals are stable and persistent environmental contaminants since they cannot be degraded or destroyed. Therefore, their toxicity poses major environmental and health problems and requires a constant search for efficient, cost-effective technologies for detoxification of metal-contaminated sites.



HEAVY METALS UPTAKE

Taking up metals is basically considered as a two-step process[9,10]. Complexation, ion exchange, adsorption, inorganic micropre-cipitation, oxidation and/or reduction have been proposed to explain the uptake process [11].

- Metal ions are adsorbed first to the surface of cells by the interactions between the metal ions and metal-functional groups such as carboxyl, phosphate, hydroxyl, amino, sulphur, sulphide, thiol etc. present in the cell wall and then they penetrate the cell membrane and enter the cells [12]. When the extracellular concentration of metal ions is higher than that of intracellular, metal ions can penetrate into the cell across the cell wall and in fact several possible mechanisms have been suggested to underline their transport [13].
- Molecular mimicry is one of such mechanisms whereby metal ions either compete for binding to multivalent ion carriers or, after binding to low molecular weight thiols (such as cysteine), enter the cell by active transport.
- In another type of mechanism, metal ions bound to chelating proteins (such as metallothioneins) may enter the cell by endocytosis [13,14].
- Metal ions can also enter the cells if the cell wall is disrupted by natural or artificial force [12]. After entering, the metal ions are compartmentalized into different subcellular organelles.

Plants have developed a number of strategies to resist the toxicity of heavy metals, such as efflux-pumps [15], complexation of heavy metals inside the cell by strong ligands such as phytochelatins [16] or histidine [17] and several other mechanisms[18].

BIOACCUMULATION

Heavy metals present in a bioavailable form may be bioaccumulated and thereby detrimentally affect organism health. Vijver *et al.* (2004) summarized the accumulation strategies in which essential and non-essential metal ions may undergo different processes. However, it seems that the intracellular accumulation is an energy driven process dependent on active metabolism. Despite the fact that many parameters play roles in the process of accumulation [19], it is clear that different species of algae accumulate heavy metal ions to various degrees. Plants that actively prevent metal accumulation inside the cells are called excluders; these represent the majority of metal-resistant plants [20]. Other resistant plants deal with potentially toxic metals in just the opposite way, i.e. they actively take up metals and accumulate them. These plants, which have been named “hyper accumulators” are able to accumulate several percent metals in the dry weight of their above ground parts [21]. The active accumulation in the above ground parts of hyper accumulator plants provides a promising approach for both cleaning anthropogenically contaminated soils (phytoremediation) and for commercial extraction (phytomining) of metals from naturally metal-rich (serpentine) soils [22].

DIFFERENT HEAVY METALS TOXICITY TO MICROALGAE

Toxicity of a metal seems to be related to cell surface interactions or to intracellular accumulation [23]. In the case of algae, toxicity primarily results from metal binding to sulphydryl groups of proteins or the disruption of protein structure or displacement of an essential element [24].

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Heavy metal ions can cause plasma membrane depolarization and acidification of the cytoplasm [25]. In fact, membrane injury is one important effect of heavy metal ions that may lead to the disruption of cellular homeostasis. A chain of metabolic events, beginning with the respiration, photosynthesis and continuing with uptake and assimilation of nutrients, dilution of intracellular level of the heavy metal ions, *etc*. seems to play an important role in balancing the cellular homeostasis, regardless of whether they are strongly or weakly correlated with the algal growth [26]. Membrane injuries seem to be common in cyanobacterial response to metal toxicity [27].

In addition, heavy metal ions could interrupt routine metabolic processes by competing for the protein binding sites; activate enzymes and various biological reactive groups, causing poor or no growth. The presence of heavy metal ions in the growth medium could induce the activity of the peroxidase that is involved in the degradation of indole acetic acid (IAA), a hormone widely known for its ability of stimulating plant growth and multiplication. Some heavy metal ions may inhibit enzymes in the cytoplasm such as esterase and b-D-galactosidase [28].

Most of the studies with microalgae (*Chlorella*, *Chlamydomonas*, *Scenedesmus* and *Pseudokirchneriella* sp.) have shown that the increase of metal toxicity with the increase of pH is a result of decreased competition between the metal ion and H^+ at the cell surface [29]. However, some studies have shown that the increase of metal toxicity with the decrease of pH is due to the predominance of the free metal ion at low pH [30].

Heavy metal ions (such as Pb^{2+}) are capable of binding to thylakoid membrane resulting in the alteration of the ultrastructure of thylakoids, which would eventually deteriorate the routine functions of thylakoids[31]. Biosynthesis of phycocyanin and carotenoid could also be affected by the heavy metal ions [32].

On the other side, Sabnis et al. (1969) attributed that chlorophyll damage on the thylakoid membranes could be due to the affinity for heavy metals. In a manner that photosynthesis was generally enhanced by low concentrations of wastes and inhibited by high concentrations.

The response of respiration was quite similar to that of photosynthesis. Singh et al. (1987) reported that the addition of Ni_2, Hg_2 and Cd_2 inhibited the growth, oxygen evolution and oxygen uptake in the cells of *Cylindrospermum*. Also, Takamura et al. (1989) stated that, cyanophyceae are sensitive to Cu_2, Cd_2 and Zn_2 metals than other algae tested for photosynthetic activity, through the inhibition of photosystem II and or reduction the four enzymes involved in the fixation of CO_2 for at least the first 2 days of the exponential growth [24].

Cadmium and Zinc

For a long time, cadmium has been known as a highly toxic metal that represents a major hazard to the environment. Only recently, results from oceanographic research have shed a new light on the environmental role of Cd. Initially it was found that the concentrations of Cd in the oceans follow a pattern that is generally characteristic of micronutrients, and not toxic substances. The uptake of Cd into the plant seems to occur via various channels for the transport of other divalent cations, in particular Zn [33]. A channel which took Cd but not Zn was detected by in the Gangese cotype of *T. caerulescens*, but later results indicated that this is the iron transporter IRT1, which had previously been shown to transport Cd as well.

As a trace element, zinc only becomes toxic to organisms above certain concentrations, in the range of micro or millimolar. This is the case in waters of many metal contaminated environments.

AE El-Enany *et al* 1999 reported that Zinc accumulation was increased as wastewater level rose in the culture medium. The zinc uptake by *N.linckia* was obviously accelerated than those of *N.rivularis*. *Nostoc linckia* accumulated about 30 fold of Zn (12.6 mg ml 1 culture) than those of growth medium (400 mg) while *N. rivularis* accumulated (5.46 mg Zn ml 1 culture) only two fold of zinc than those of waste water. About 60–65% of Cd or Zn was retained by sediment (pellets), the remainder was found in the cytosolic fraction (the supernatant). These results are in accordance with those of many investigators[34].

Cyanobacteria have a remarkable affinity for heavy metals. Metallothioneins are known for detoxification ofmetal ions in animals and fungi [34-36]. Ma Clean et al. (1972) was the first who reported that presence of Cd-binding material in a fresh blue-green algae (eg. *Anacystis nidulans* and he found that cyanobacterium (*Anabaena dolilolum*) synthesized low molecular weight Cd-binding protein (3.3 kDa) in response to Cd and they concluded that, this protein may play a role in metal tolerance. Also, Bierkens et al. (1998) concluded that HSP (70 kDa) was induced in grown algae as a response of heavy metal pollutants. In this respect Torres et al. (1997) found that marine algae, in response to Cd synthesized metallothioneins which sequester the metal in harmless form.

Occurring of these metal-binding in organisms growing in a mining refuse area also support the postulate that they are involved in detoxification [37].

Copper

Wilde *et al*. (2006) reported that copper has no effect on other cell functions such as photosynthesis, respiration, ATP production, electron transport and membrane ultra structure, though it inhibits the cell division of *Chlorella* sp. How-ever, Wong *et al*. (1994) reported copper-

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induced structural alterations in thylakoid membranes of *Chlorella* sp. and inhibition of photosynthesis.

PHYTOREMEDIATION OF HEAVY METALS BY MICROALGAE AND ITS POTENTIAL APPLICATION

Heavy metals have been released into the environment over long periods of time, throughout many activities of man. Once the metals have been released into the environment, they are difficult to be removed by physical or chemical means and most of them exhibit toxic effects on organisms. In addition, conventional physicochemical means for removing heavy metals from wastes are generally very expensive [38]. Accordingly, a great deal of interest has recently been generated in using microbes as biosorbents for metal removal. Algae represent the best biological treatment for wastewater because they increase O_2 content of waters v*ia* photosynthesis and sorption of some heavy metals contaminated waters by reducing the cellular antioxidant capacity [39].

The selection of such organisms is explained by the many metal-detoxification or metal-controlling mechanisms found. According to Simkiss (1993), one of the abilities of living systems are to have cells capable of regulate and compartmentalise ions from their surroundings, and this would lead to biomineralization towards the production of amorphous minerals. Among them there are granules containing phosphorus, calcium and magnesium. Their amorphous structure is important for both presumable functions of stocking and detoxifying ions [40]. Many heavy metal ions have a direct influence on various physiological and biochemical processes of microalgae. As the growth reflects the metabolism of the cell, it has been used as a key indicator of the toxicity of heavy metal ions in microorganisms and it depends on the proper functioning of various metabolic processes, such as photosynthesis, respiration and nutrient uptake, etc [26]. Growth inhibition in microalgae is related to the amount of heavy metal ions bound to the algal cell surface. The process basically involves physiochemical and biological approaches.

Physicochemical approaches

Heavy metal removal mechanisms include sedimentation, flocculation, absorption and cations and anion exchange, complexation, precipitation, oxidation, reduction, microbiological activity and uptake. These methods often lack the specificity required for treating target metals. They are also inefficient and expensive, especially in cases where metal concentration in the wastewater is low. In addition, high cost and complicated operation often limit their use in large-scale *in situ* operations [41-42].

Biological approaches

Biological approaches are based on the use of naturally occurring processes. Many microorganisms take part in the biogeochemical cycling of toxic heavy metals. Microalgae and other microorganisms play a significant role in the transformation of heavy metal ions in the environment[43,5]. Organic compounds released from growing cells, as well as biodegradation products of various origins, may serve as complexing agents for metal ions, thereby decreasing metal toxicity [22,44-45]. The binding of metal ions to cell wall components of microalgae was also reported [44,46]. Various metabolic processes such as photosynthesis, respiration, and nutrient uptake take place during the growth of microalgae. All influence the equilibrium between free metal ions and the bound forms, as well as that between sedimentation and redissolution in the aquatic environment. Once entering the cell, the heavy metal ions may either be detoxified or adversely affect cell processes such as photosynthesis and cell division [47]. Microalgae thriving in metal contaminated sites also possess intracellular mechanisms that enable them to cope with the toxic effects of metals [48-49]. Such species may be used for *in situ* bioremediation of large water bodies contaminated with low concentrations of metal ions (for more detailed comparisons between physicochemical and biological approaches for metal detoxification.

To date, it is generally accepted that technologies based on naturally occurring biological processes have a number of advantages over presently available physicochemical techniques for remediation of sites contaminated with toxic heavy metals.

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[50-51]. The potential of many organisms (algae, bacteria, cyanobacteria, fungi, and plants) as well as dead biomass derived from them for metal bioremediation was examined [3].

Microalgae are very abundant in the natural environment and are well adapted to a wide range of habitats for example fresh- and seawater, domestic and industrial effluents, salt marshes, and constructed wetlands. They have a remarkable ability to take up and accumulate heavy metals from their surrounding environment. Their ability to sequester various metal ions such as copper, cadmium, nickel, gold and chromium is well documented[21,52]. Therefore, attempts were made to use microalgae, living cells or their dead biomass for removing heavy metals from contaminated waters [52-53]. The use of living cells is most efficient for removal of metal ions from large water bodies containing low concentrations (ppb range) of metal ions.

Thus, living prokaryotic and eukaryotic microalgae can be used as a complementary treatment step, following physiochemical processes which are applied in sites containing high metal concentrations. Resistant microalgal species isolated from metal contaminated sites have a higher capacity for accumulating heavy metals compared with species isolated from non contaminated sites [54-55]. During algal growth, metals are removed from the surrounding environment and accumulated in the cells by both nonmetabolic-dependent processes

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(adsorption) and metabolic-dependent ones (absorption) [56,48]. Biosorption of heavy metals by living immobilized prokaryotic and eukaryotic microalgae cells, using various immobilizing material, is an additional option. Generally immobilized cells are more efficient in the removal of heavy metals compared to free living cells. In addition, by using immobilized cells harvesting of the algal biomass is more efficient [57-59,23]. This can be achieved by providing adequate environmental conditions for supporting microalgae growth, such as light, temperature, and pH are present, the use of living microalgal biomass offers anefficient, simple, and cost-effective method. Microalgae in consortium with other microorganisms, such as microbial mats are also capable of removing metals and metalloids as well as other recalcitrant organic compounds from contaminated sites [60-62]. Microalgal biomass has been successfully used as sorbing material [63-65]. The vast majority of the studies were conducted with synthetic solutions containing single metalion and only limited information is available on biosorptionby active (living cells) or inactive (nonliving cells)prokaryotic or eukaryotic biomass exposed to a mixture of several metals simultaneously. In a few studies, the effect of dissolved organic matter on metal speciation and detoxification is also addressed. Moreover metals adsorbed on cell wall surfaces of algal biomass can be recovered and the sorbing material can be restored for reuse [66]. Removal of metals from sites contaminated with high concentrations of metals can be achieved using non viable biomass as biosorbents. Yet, it should be noted that biomass obtained from different algal species differ largely in their binding capacity for various heavymetals[67].

Metal detoxification mechanisms as metallothionein and polyphosphate granules

Various mechanisms such as production of heavy metal binding factors and proteins (metallothionein, GSH and phytochelatin conjugates), exclusion of toxic heavy metals from cells by ion-selective metal transporters and excretion or compartmentalization have been proposed for reducing heavy metal toxicity to [68-69]. Additional function of metallothionins include control of intracellular redox potential, cellular repair processes, growth and differentiation, where they are likely to serve as the source of Zn for newly synthesized apoenzymes, as well as regular molecules in gene expression.

Although a polyphosphate granules method is less sophisticated, seems to be more effective than a metallothionein, which is sometimes very specific for binding only one metal. The non-specificity of the granules gives them the ability of binding many different metals. Polyphosphate granules are a common structure in cyanobacteria [70-25]. They are the bacterial counterparts for the phosphorus granules and probably these two structures have some similar functions in cells. The elemental composition of the polyphosphate granules of cyanobacteria is usually phosphorus, magnesium, potassium and calcium sometimes, sulphur is also present [71-72] and zinc is present in the polyphosphate granules of the cyanobacterium *Synechocystis aquatilis*. Besides, it was also shown show that increase in the number of glycogen granules in these cells.

Sorption of heavy metals on phytoplankton cell surfaces is dependent on a number of factors ranging from the concentration of inorganic ions, dissolved organic matter, pH and the nature of particulates [73-74]. The extent of sorption and uptake of trace metals is expected to vary in algal cell surface characteristics and in the physiological state of the algae [75]. The two heavy metals Cd and Zn are well known fresh water pollutants. Adsorption equilibrium constants for Zn were measured 0.123 and 0.039 mmol for *Scenedesmus subspicatus* and *Chlamydomonas variabilis* respectively [76]. Cadmium was found also to be accumulated by various green algae in variable amounts [77].

CONCLUSION

It could be suggested here that heavy metal ions can inhibit the growth of microalgae in different ways, which depend on the species, the metal types and the condition in the growing media. In conclusion, many species of prokaryotic and eukaryoticmicroalgae, as well as their inactive cell biomass, can be used for bioremediation of metal-polluted sites. In order to bring this potential to the applicable stage on a commercial basis; more information on metal detoxification efficiency upon exposure of microalgae biomass to various metal-contaminated effluents is required. Such effluents usually contain a mixture of inorganic and organic compounds which might affect metal speciation and their availability and therefore influence the efficiency of the detoxification processes. So far most experiments were made in laboratory scale reactors thus treatment of large volumes of contaminated sites requires up scaling. To achieve this target, interdisciplinary cooperation among professionals from various fields, for example, biologists, chemists, engineers, and environmentalists, would be fruitful. Hence, we suggest further investigations on the role of polyphosphate granules of this cyanobacterium in the presence of these other pollutants of the bay. Algae are predominantly aquatic organisms that must be able to discriminate between essential and non-essential heavy metal ions. In addition, they must maintain nontoxic concentrations of these ions inside their cells. In this way, two principal mechanisms have been identified, one which prevents the indiscriminate entrance of heavy metal ions into the cell, i.e., exclusion, and the other which prevents bioavailability of these toxic ions once inside the cell, i.e., the formation of complexes. Extensive surveys of heavy metal tolerant algae are needed in order to obtain new data that verify and increase current knowledge of the mechanisms involved.

Acknowledgement: We are highly thankful to Maharshi Dayanand College, Sriganga nagar (Raj.) for providing praise worthy contribution to prepare this paper.

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Source of Support: Nil, **Conflict of Interest:** None.

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