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Bioremediation of heavy metal pollutant Lead by Microalgae and its impact on their growth and Chlorophyll-a content : A Review

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ABSTRACT

Heavy metals are major pollutants of wastewater that cause significant changes in various parameters in water bodies. Increased concentration of these pollutants causes changes in water quality and ultimately affects living organisms. Microalgae can be considered as an effective bioremediation tool for the removal of these heavy metals due to their fast growth rate, simple methods of culturing, ecological sustainability and capability to adapt in various changing environmental conditions. Lead is one of the toxic heavy metals which causes harmful effects to aquatic animals as well as human health. This metal has no significant biological function and hence gets accumulated in microalgal cells, subsequently getting transferred to other organisms through food chain. It is important to study the effects of Lead toxicity on various aspects related microalgae which can affect the survival of these organisms. Though microalgae are an effective biosorbent, after a certain limit of Lead concentration, their internal protective system can collapse which can result in a drastic decline in their growth rate and also cause harmful effects on their photosynthetic pigments. This paper gives a review on bioremediation of heavy metal Lead using microalgae and also the impact of this process on growth and chlorophyll-a content of microalgae.

Keywords: Microalgae, Bioremediation, heavy metal Lead, ecological sustainability, chlorophyll-a.

INTRODUCTION

Water pollution due to heavy metals has become one of the major global issues due to the persistent natureof heavy metals, high toxicity, bioaccumulation in organisms, biomagnification across food chains and the resulting harmful effects. Most common pollutants are Mercury, Lead, Cadmium, Zinc, Copper, Nickel, Arsenic etc. (Sibi, 2019; Wilson *et al*., 2015). These heavy metals usually reach the water bodies through contaminated surface water, ground water or industrial waste water. As high concentration of these heavy metals causes harmful effects on aquatic animals as well as on humans, exposure to Lead too shows various harmful effects on plants, animals as well as on humans (Tiwari , 2013; Dewi *et al*., 2018), as this toxic heavy metal has no significant biological function, it accumulates in aquatic plants and is subsequently transferred to other organisms through food chain (Souza *et al*., 2012).

Heavy metals are not easily degradable and are also highly toxic, hence it is necessary to develop significantly effective techniques to remove these pollutants from water. Currently there are several technologies, which have been used, such as precipitation, adsorption, reduction, coagulation, membrane filtration etc. however, they have several drawbacks such as, they are costly and can produce secondary waste which requires further disposal techniques and thus not so environment friendly. Also some of them prove non effective in removal of pollutants that exist in trace amounts (Sibi, 2019).

Research data available till now has demonstrated that bioremediation could be a promising and cost-effective technology for the removal of heavy metals wherein microorganisms can be successfully utilised for biosorption of these metals from polluted waters (Redha, 2020; Beni, 2020; Kanamarlapudi ,2018). In comparison to the conventional methods of heavy metal removal, biosorption by microorganisms has several advantages such as it is cost effective, environmental friendly, can remove heavy metals even if they exist in trace amounts. (Pacheco *et al*., 2020). Various biosorbents have been used so far for biosorption of heavy metals which include Microalgae, Bacteria, Fungi, Macroalgae, Molluscan shells,etc. However, microalgae are particularly attractive due to their availability in large quantities in many regions of the world, can grow easily even in waste waters and in various climatic conditions, have low culturing costs and have an excellent retention ability for most toxic heavy metals (Prabha *et al*., 2016).

Though microalgae serve as quite an effective biosorbent for various heavy metals including Lead, excessively high concentration of Lead can show drastic effects on their ultrastructure and biological functions which can further affect the organisms at higher trophic levels as microalgae are primary producers of the aquatic ecosystems (Arunkumara, 2008). The purpose of this review is to throw light on microalgal species effectively utilized for bioremediation of Lead and also on some aspects of Lead toxicity in these organisms; specifically, the effect on growth and chlorophyll a content of microalgae being used in Lead bioremediation. These parameters are essential to be studied when considering the effects of toxic substances, because they are vital requirements for functioning of important processes necessary for survival of these organisms. Also as microalgae are the primary producers of the aquatic ecosystems, effect on their population will ultimately affect the organisms which are dependent on them including humans.

Microalgae: Ecological and Commercial Significance

Marine Microalgae are world's largest group of primary producers of aquatic environment, responsible for at least 32% of global photosynthesis. They, thus play an important role in CO_2 recycling through photosynthesis and liberating oxygen (Matsunaga *et al*., 2005). They are widely distributed all over the world. They are unicellular, microscopic phytoplanktons having a size of 2-20 µm. Microalgae can grow alone or in symbiosis with other organisms. Microalgae perform photosynthesis by using sunlight and CO_2 from the atmosphere and convert them into organic carbon to obtain energy. They are thus good sources for CO_2 fixation and liberation of O_2 into the atmosphere. Biodiversity of Microalgae includes eukaryotic microalgae such as green microalgae, red microalgae, diatoms and dinoflagellates as well as prokaryotic cyanobacteria (Miazek, 2015). Microalgae are able to adapt in various extreme conditions. Microalgae found in coastal regions are able to thrive in turbulence, high nutrients and low light while open ocean microalgae are adapted to high irradiance and low nutrients. Microalgae found in the polar regions are able to survive in freezing temperatures as well as long periods of light and darkness. Because of ability to adapt to various conditions, microalgae have become a dominant population of aquatic ecosystems (Hopes *et al*., 2015).

Valuable compounds such as lipids, pigments, carbohydrates, vitamins, and proteins can be obtained from microlgae and they also have potential applications in many industries (Miazek, 2015). Photosynthetic pigments such as chlorophylls, carotenoids and phycobiliproteins are also used in food, cosmetics, pharmaceutical products, colouring agents, antioxidants, food additives or therapeutic agents. Cyanobacteria can produce a variety of biochemically active compounds such as enzyme inhibitors, herbicides, antimycotics, antifeedants, antimalarial, multi-drug resistance reversers, immuno-suppressive agents, etc. Polysaccharides produced by red algae have commercial potential (El Gamal, 2010). Chlorophyta is used in food additives. Commercial cultivation of *Dunaliella* has been undertaken for food supplements and β-carotene production. Few strains of *Rhodomonas minuta* and *Cryptomonas* sp. contain significant amounts of poly- unsaturated fatty acids (PUFAs) hence they used as aquaculture feed. Euglenophytes play an important ecological role in specific marine environments such as, *E. gracilis* generates antioxidant vitamins like β-carotene and vitamins C and E (Matsunaga *et al*., 2005).

Haptophytes such as *Isochrysis galbana* and *Pavlovai*, are used as living feeds for bivalve molluscs and crustacean larvae. About 60 dinoflagellate species produce cytolytic, hepatotoxic or neurotoxic compounds and thus can be studied further to be utilised as biopesticides in future (Priyadarshani *et al*., 2012; Matsunaga *et al*., 2005). *Crypthecodinium cohnii* produces DHA which widely as food supplements. Five important Pinguiophyceae species that have been widely used as fish feed and for Polyunsaturated Fatty acids (PUFA) production are *Pinguiochrysis pyriformis, Phaeomonas parva, Polypodochrysis teissieri, Pinguiococcus pyrenoidosus* and *Glossomastix chrysoplasta* (Matsunaga *et al*., 2005). Docosahexaenoic acid (DHA) from *Schizochytrium* sp., Eicosapentaenoic acid (EPA) from *Chlorella minutissima,* Arachidonic acid (AA) from *Parietochlorisincise*, Biotin from *Euglena gracilis* etc. are some high-value bioproducts which are extracted from microalgae. These organisms also have application in biofuel, biofertilizer and aquaculture feed production (Priyadarshani *et al*., 2012). *Pavlova, Isochrysis, Phaeodactylum, Nannochloropsis, Chlorella, Thalassiosira, Tetraselmis, Skeletonema* and *Chaetoceros* are most commonly utilized as a feed in aquaculture. Because of all these applications, microalgae cultivation can be a profitable business in the biotechnological industry (Matsunaga *et al*., 2005). In many cases, it was observed that the production yield of extracellular polymeric substances (EPS) and carotenoids from microalgae is much higher than bacteria which makes them the most cost-effective option over bacteria. Further microalgae-derived bioactive compounds show a higher bioactivity rate as compared to marine-bacteria derived compounds (Hamidi, 2020).

The need to curb Lead pollution

Lead (Pb) is one of the major toxic heavy metal found in industrial waste and agricultural waste which is discharged into water bodies. Important sources of this naturally occurring toxic metal are mining, smelting, manufacturing, recycling activities also leaded paints, leaded gasoline, lead-acid batteries, solder, stained glass, ceramic glazes, jewellery, crystal vessels etc. Mining and smelting processes for Lead are responsible for releasing air pollutants such as sulphur dioxide, nitrogen oxides etc. (Tiwari, 2013; Singh *et al*., 2014). Volcanic explosions and forest fire too are natural sources of Lead (Zhang *et al*., 2015). Lead is majorly used in making rechargeable storage batteries. Manufacturing of lead-acid batteries for motor vehicles is said to be the cause of more than three quarters of global Lead consumption. Tetraethyl Lead is one of the most important organic compounds of Pb, which is a very efficient and cheap antiknock additive to gasoline hence widely used to make pipes and reaction tanks (Singh *et al*., 2014; Zhang *et al*., 2015)

The presence of Lead in higher concentration can result in adverse effects such as the loss of biodiversity, community components alteration, decreased seed viability and pollen germination, reduced growth and reproductive rates in plants and animals (Zhang *et al*., 2015). High exposure to Lead is hazardous to human health which attacks the brain and central nervous system to cause coma, convulsions and even death (Singh *et al*., 2014). Lead gets accumulated in the developing muscles, bone and brain connections of children that causes a rise in blood pressure, kidney damage, brain damage, affects the learning ability of children, behavioural disruptions, decline in fertility of men through sperm damage, etc. (Reyes, 2015; Tiwari, 2013). The concentration of Lead near the roadside soils is found to be considerably higher than soil that is farther from the roads which indicates that motor vehicle pollution is one of the sources of Lead pollution. Lead present in soil can cause poisoning as it gets accumulated in plants growing in that soil and also through run off of surface water, may end up in water bodies (Tiwari, 2013). Some other sources of Lead such as plumbing material and solder also contaminate drinking water (Zhang *et al*., 2015; Tiwari *et al*., 2013). The effect on microalgae population because of the heavy metals can show serious implications on water quality as well as on higher trophic organisms as heavy metals have ability to accumulate in the biota (Ebenezer and Jang-Seu Ki, 2012).

Bioremediation of heavy metal pollutant Lead

Bioremediation of Lead by microorganisms significantly involves the mechanism of biosorption which is a physiochemical process that naturally occurs in specific biomass that passively concentrates and binds the cellular structure with contaminants present in waste water. Biosorption is a highly cost-effective and eco-friendly method for the removal of heavy metals. A wide variety of active and inactive organisms have been utilized as biosorbents for the removal of heavy metal ions from aqueous solutions (Ubando *et al*., 2020).

Shells of Anodontoides ferussacianus (Paper shell mussel) can uptake upto 200 mg/L of lead at 15 g/ L of biomass concentration (Shahzad *et al*., 2017). Dried biomass of aquatic plants such as *Pistia stratiotes* (water lettuce), *Eichhornia crassipes* (water hyacinth) and *Ipomoea aquatic* (water spinach) are also used as effective biosorbents, showing biosorption efficiency of about 62.71%, 95.56% and 98.30% respectively (Visperas *et al*., 2020). Various marine macroalgae have been utilised for the removal of heavy metals for eg. *Eucheuma spinosum, Padina minor, Sargassum crassifolium, Ulva fasciata, Sargassum filipendula* etc. which are known to show about 96-97% Lead uptake from surroundings (Putri, 2016; Nessim *et al*., 2011; Costa *et al*., 2007). Some bacteria such as *Bacillus* sp. and fungi such as *Penicillium* sp. have been effectively used as biosorbents for Lead removal (Ezzouhri *et al*., 2010; Varghese *et al*., 2021).

Microalgae involved in bioremediation of heavy metal pollutant Lead

Marine algal biomass is observed as a promising biosorbent, due to its high uptake capacities, low cost, renewability, simplicity of cultivation, the capability of adaptation to various changing environmental conditions as well as the ready abundance of the biomass in several regions of the world (Prabha *et al*., 2016). Microalgae such as *Tetraselmis marina, Stigeoclonium tenue* and *Spirogyra* sp., have shown potential in metal pollution bioremediation (Pacheco *et al*., 2020; Prabha *et al*., 2016). *Chlorella vulgaris, Chlorella marina* are used in the removal of Lead and Chromium, from textile waste effluents (Kassas and Mohamed, 2014; Kumar *et al*., 2015). Alginate is a polysaccharide constituent present in *C. vulgaris*, which can bind with Pb so the toxicity level decreases for the nearby organisms (Dewi *et al*., 2018). *Nannochloropsis oculata* can efficiently remove about 55% Lead at 1.3 ppm concentration of Lead while at 0.5 ppm it could be 28% (Waluyo *et al*., 2020). *Ecklonia radiata* can remove Lead even when it is in very low concentrations and also, in comparison to wood rotting fungus (*Phellinus badius*), wood bark (*Pinus radiata*) and yeast (*Saccharomyces cerevisiae*), it showed much higher uptake of Lead from waste water (Matheickal and Qiming Yu, 1996). Table 1 gives some more details on species of Microalgae which have been successfully utilized in bioremediation of Lead.

Microalgae have been used in bioremediation because they possess the potential to tolerate heavy metals, also they maintain high yields of recovery per unit mass and they contain a cell wall which is loaded with ionisable groups. For the intracellular heavy metal detoxification, microalgae form metal-binding proteins which are class III metallothioneins (MT) or phytochelatins (PC) (Monteiro *et al*., 2011).

Impact of Lead on growth and chlorophyll-a content of Microalgae

Increased concentration of Lead highly influences the morphology and biological functions of microalgal cells which ultimately affects the growth and chlorophyll content of microalgae (Carfagna *et al*.,2013). If the metal concentration is high then it may inhibit cell growth because the organism's internal protection system will no longer be able to tolerate the effects of toxic metals which leads to inhibition of biological functions of microalgae (Dewi *et al*., 2018). Chlorophyll-a pigments provide an effective index of the photosynthetic capability of a population (Priyadarshani *et al*., 2012; MacIntyre *et al*., 1996). High concentration of Lead in the surroundings can cause excessive bioaccumulation of Lead in microalgae which is responsible for the production of Reactive Oxygen Species (ROS) because it can strongly bind to amino acids, DNA, enzymes and RNA and cause their degradation. Increased ROS causes increasing membrane lipid peroxidation and permeability in cells (Singh *et al*., 2014, Miazek, 2015). Lead can alter the absorption of Mg and Fe which reduce the synthesis of chlorophyll pigments. Hence, high concentration of Lead can affect the microalgal morphology, photosynthesis and thus impacts their growth (Souza *et al*., 2012).

Table 1 Some Microalgal species involved in bioremediation of Lead

Microalgae	Bioremediation efficiency with respect to heavy metal Lead	References
Spirulina sp.	In the initial stage (0-12min) the adsorption rate was 74% and its biosorption capacity was estimated to be 0.62 mg Lead /10^5 algal cells.	Prabha *et al*., 2016
Dunaliella sp.	Can tolerate high concentration of heavy metals and it has a great ability to remove metals from aquatic environments.	Prabha *et al*., 2016
Chlorella marina (Butcher)	Seven days incubation of cells in waste water during which they increased from $3x10^6$ to $1.5x10^7$ cell/ml and reduced Lead content upto 87%	Prabha *et al*., 2016
Chlorella vulgaris	Dried dead biomass was studied in terms of its performance in binding divalent Pb ions from aqueous solutions. Lead ions were found to decrease with increase in donor numbers	Prabha *et al*., 2016
Chlorella vulgaris	Metal uptake- 97.38 mg/g of biomass	Kumar *et al*., 2015
Phaeodactylum tricornutum	Metal uplift $0 \rightarrow 10$ µM and toxic effects avoided	Miazek *et al*., 2015
Chaetoceros sp.	Maximum biosorption-8 mg/g at 20 mg/L of Pb	Ubando *et al*., 2021
Immobilized Chlamydomonas reinhardtii	Maximum biosorption- 308.7 mg/g at 500 mg/L Pb	Ubando *et al*., 2021
Phormidium sp.	Maximum biosorption- 2.305 mg/g at 10 mg/L Pb	Ubando *et al*., 2021
Rhizoclonium hookeri	Maximum biosorption-81.7 mg/g of biomass	Ubando *et al*., 2021
Anabaena flosaquae	Metal uptake – 70 mg/g of biomass	Kumar *et al*., 2015
Arthrospira (Spirulina) platensis	Metal uptake- 102.6 mg/g of biomass	Kumar *et al*., 2015
Aulosira fertilissima	Metal uptake- 31.12 mg/g of biomass	Kumar *et al*., 2015
Calothrix parietina TISTR 8093	Metal uptake- 45 mg/g of biomass	Kumar *et al*., 2015
Chlamydomonas reinhardtii	Metal uptake- 96.3 mg/g of biomass	Kumar *et al*., 2015
Cyclotella cryptica	Metal uptake- 36.68 mg/g of biomass	Kumar *et al*., 2015
Hydrodictyon reticulatum	Metal uptake- 24 mg/g of biomass	Kumar *et al*., 2015
Microcystis novacekii	Metal uptake- 80 mg/g of biomass	Kumar *et al*., 2015
Oscillatoria laete-virens	Metal uptake- 21.6 mg/g of biomass	Kumar *et al*., 2015
Phaeodactylum tricornutum	Metal uptake- 1.49 mg/g of biomass	Kumar *et al*., 2015
Phormidium sp.	Metal uptake- 13.6 mg/g of biomass	Kumar *et al*., 2015
Pithophora odeogonia	Metal uptake- 71.13 mg/g of biomass	Kumar *et al*., 2015
Porphyridium purpureum	Metal uptake- 0.32 mg/g of biomass	Kumar *et al*., 2015
Pseudochlorococcum typicum	Metal uptake- 4.49 mg/g of biomass	Kumar *et al*., 2015
Scenedesmus acutus IFRPD 1020	Metal uptake- 90 mg/g of biomass	Kumar *et al*., 2015
Scenedesmus subspicatus	Metal uptake- 38.71 mg/g of biomass	Kumar *et al*., 2015

Most reports demonstrated that with an increase in metal ion concentration, their inhibitory effects on biological functions are also higher thus leading to reduction in the growth rate of algae due to a decrease in algal photosynthesis caused by the inhibition of synthesis of chlorophyll, as it is the most important pigment in algal cells which responsible for photosynthesis (Al-Hejuje, 2008).The accumulation of Lead in *Chlorella sorokiniana* culture leads to misshaping of chloroplasts (Miazek, 2015). Pb can alter the chlorophyll-a content and also can inhibit the growth and enzyme activity of algal cells (Gan *et al*., 2019). Microalgae react to metal-induced oxidative stress by producing chelating agents such as phytochelatins or exopolymers, which forms chelate–metal coordination complex, that helps to prevent metal ions interaction with biological macromolecules (Miazek, 2015). However, in case of high concentration of metal ions, heavy metal binding to significant macromolecules cannot be prevented and thus it interferes with several normal functions of microalgae and also causes oxidative damage (Carfagna *et al*., 2013).

The heavy metals can cause membrane depolarization and acidification of the cytoplasm and also the membrane injury that may lead to disruption of cellular homeostasis (Moh. Muhaemin, 2004). Lead can replace Mg^{2+} ion in the centre of chlorophyll structure resulting in a decrease photosynthesis rate (Miazek, 2015). The effect of heavy metal on photosynthesis could be the inhibition of the PS II which leads to the damage of thylakoid membranes and reaction centres. It can also affect the ultrastructural alteration in the shape and organization of thylakoids. The decrease in chlorophyll level indicates the disruption in the photosynthetic apparatus of microalgae (Carfagna, 2013). In other studies of cultivation of marine dinoflagellate *Cochlodinium polykrikoides*, the initial concentrations of Lead which were 0.05-10 mg/L indicated no significant change while those above 25 mg/L concentration showed a decrease in the cell counts and Chllrophyll-a level which was almost 75% (Ebenezer, 2012). Lead showed aggravated effects on *Synechocystis* sp. PCC 6803 when cultivated in different concentrations, one of them being the large empty spaces in the cell interior which were the result of thylakoid destruction in chloroplasts of these organisms (Arunakumara, 2009).

In a study where microalgal cultures were incubated with Lead, a decrease in growth rate was observed as incubation progressed. Compared to the Control, the inhibitions at the end of 6 day incubation were 8.4, 12.4, 13.9, 29.1, 42.9 and 47.6% respectively for 0.5, 1, 2, 4, 6 and 8 mg/L of Lead (Pb). While pigment contents such as chlorophyll-a, β-carotene and phycocyanin reduced 36.56, 37.39 and 29.34% respectively with the increased metal concentration at 6 mg/L Pb over the control (Arunkumara, 2009). In another study for physiological and morphological responses given by *Chlorella sorokiniana* 211-8K to Lead toxicity, upto 77% decrease in the rate of photosynthesis was observed on treating it with 250 µM Pb for 24 hours, however, respiration increased upto 300%. Exposure to Pb induced a reduction in the content of the total chlorophylls and the soluble protein level. Increased exposure to heavy metals affected the overall ultrastructure of the chloroplast of microalgae (Carfagna *et al*., 2013). Effects of different concentrations of Lead towards *Nostoc linkia* and *Hapalosiphon aureus* for 4 weeks showed, increased chlorophyll pigments at 1mg/L and 10mg/L treatments at 2nd and 3rd weeks of incubation respectively in *N. linkia* while in *H. aureus* growth was inhibited at the first week of exposure for all concentrations (Al-Hejuje, 2008). Maximum reductions of chllorophyll-a and β carotene of *Spirulina (Arthrospira) platensis* were 67% and 53% respectively when treated with 100 µg/L of Lead (Arunakumara, 2008).

Although heavy metals generally have negative impact on microalgae cultures, some reports also suggest their positive role during microalgae cultivation. At low concentrations, Lead had stimulatory effect on growth of *Dunaliella tertiolecta* and *Monoraphidium minutum* (Miazek, 2015).

CONCLUSION

Bioremediation using microalgae is definitely a desirable option considering their efficiency, low cost culturing and maintainence as well as their environment friendly features supporting ecological sustainability. Microalgae are capable of Lead biosorption, however, monitoring this process at regular intervals is advisable considering the observations that high exposure to Lead shows a decrease in growth rate as well as chlorophyll-a content of microalgae. This review will also serve as an important basis for consideration in the studies related to the biological toxicity of heavy metal Lead and thus will be helpful in ecological risk assessment.

FUTURE SCOPE

Microalgae are characterized by rapid growth rates and ubiquitous distribution throughout natural environments, yet they show a critical sensitivity to environmental variations eg. nutrient levels and presence of pollutants. Toxicity tests resorting to microalgae are relatively quick and inexpensive (Monteiro *et al*., 2011) and hence they can be easily utilized for testing the toxicity levels in water bodies. These organisms have been found to precipitate and bio accumulate the entrapped metal ions in different cellular organelles as a means to survive heavy metal stress. The large surface area to volume ratio and high-affinity functional groups on the cell surface of microalgae makes them susceptible for uptake and hence serves as proficient agents for metal storage systems (Rajamani, *et al*., 2007) and thus making them potential candidates that could possibly be capable of bioremediation of heavy metals from highly contaminated waters on suitable genetic alteration.

Conflicts of Interest: The authors declare that there is no conflict of interest with respect to this review paper.

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