# Chapter 4
# Efficiency of Algae for Heavy Metal Removal, Bioenergy Production, and Carbon Sequestration

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**Abstract** Environmental contamination is one of the paramount concerns engulfing the entire world. Being nondegradable in nature, heavy metals (e.g., Ni, Cd, Cu, As, Hg, and Pb) are significant pollutants of soil and aquatic ecosystems. Although numerous technologies have been employed to remove toxic metals from contaminated sites, there is still need for more efficient and ecologically sound methods. The use of algal species for the removal of heavy metals as well as other contaminants like dyes, nutrients, ions etc. from water and wastewater, which is popularly known as phycoremediation, has been found to be eco-friendly, ecologically sound, and a value-added tool. The common algal species which are being used for phycoremediation are *Chlorella*, *Scenedesmus*, *Oscillatoria*, *Lyngbya*, *Gloeocapsa, Spirulina*, *Chroococcus*, *Synechocystis*, and *Anabaena*. The use of algae for the removal of pollutants also helps in carbon sequestration and biofuel production. This chapter discusses the removal of toxic metals from contaminated aquatic ecosystems using various species of micro- and macroalgae along with factors that influence the process of phycoremediation and the role of algae in biofuel production and carbon sequestration.

**Keywords** Biofuels · Heavy metals · Microalgae · Macroalgae · Wastewater · Phycoremediation

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## 4.1 Introduction

Industrialization and urbanization have caused overload of contaminants into the environment. In the last few decades, the pollutants have led to deterioration of many natural ecosystems. Because of their nondegradable nature, heavy metals are a special category of environmental pollutants. They enter ecosystems through a number of anthropogenic activities such as mining, smelting, refining, and electroplating industries. Due to their non-degradable nature, heavy metals accumulate in the soil and sediments and bioaccumulate in the flora and fauna of aquatic ecosystems (Forster and Wase 1997; Chandra et al. 2009; Yadav et al. 2017). Various scientific methods have been investigated for the removal of heavy metals from the contaminated water bodies: physicochemical techniques like chemical oxidation/reduction, electrochemical treatment, precipitation, ion exchange, ultrafiltration, reverse osmosis, and bio-membrane (Ahluwalia and Goyal 2007; Barakat 2011; Saxena et al. 2020). However, these technologies are found to have numerous limitations such as their non-eco-friendly nature, high operational costs, low efficiency, and other concerns (Khoshmanesh et al. 1996; Chong et al. 2000).

Application of living organisms for the removal of toxic metals from contaminated water bodies has been identified as a promising alternative of these physicochemical techniques (Mishra and Bharagava 2016). The use of algae for the remediation of aquatic contaminants from water and wastewater is known as phycoremediation. Along with the removal of toxic metals, it has been observed that algae, especially microalgae, are ideal for sequestration of nitrogen (N) and phosphorus (P) from wastewater because they require these elements as growth nutrients (Mehta and Gaur 2005; Ruiz-Marin et al. 2010; Xin et al. 2010; Pittman et al. 2011; Babu et al. 2018). This chapter highlights the removal of several toxic heavy metals by various algal species and their metal removal potential along with different factors that influence the process. The potential of carbon sequestration and biofuel production as value-added properties of phycoremediation are also discussed in this chapter.

## 4.2 Sources of Heavy Metals

The origin of heavy metals can be both natural and anthropogenic and have widespread environmental distribution. Natural sources of heavy metals occur through geological and geographical processes like nonuniform formation of parent rock, stratigraphy, topography, weather, erosion, volcanic eruptions, forest fires, aerosol particulates, uptake of metals by plants, subsequent release through decomposition, and other natural sources (Fig. 4.1). In the environment, rocks and soils are the primary natural sources of heavy metals. Hazardous impacts of volcanic eruption affect the environment and health which are exposed to heavy metals. During volcanic eruption, various gases like $SO_2$, $CO_2$, and CO, various organic

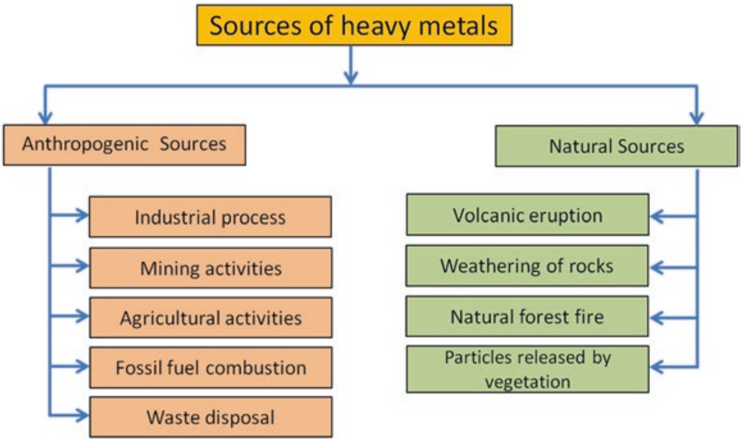

**Fig. 4.1** Sources of heavy metals in the environment

compounds (VOCs), and heavy metals like Hg, Pb, Cd, and Au are released into the environment. Various heavy metals enter vegetation through root uptake in the soil or aerial deposition onto leaves and subsequent absorption or drainage into open stomata (Sardar et al. 2013).

Anthropogenic sources of toxic metals include industrial processes; mining and extraction operations; textile activities; landfill leaches; sewage discharge; urban runoff; industrial and municipal wastewater; fossil fuel combustion; wastewater application on agricultural land; application of fertilizers, insecticides, and pesticides; and other human sources (Morais et al. 2012). The main heavy metals found at polluted sites are Cd, As, Cr, Pb, Cu, Zn, Hg, and Ni (Wuana and Okieimen 2011; Bharagava and Mishra 2018).

Arsenic is a naturally occurring metalloid. Arsenic rarely exists as a free element in nature, but rather as a powdery amorphous and crystalline form in ores. The natural sources of arsenic are contributed by weathering of rocks, forest fires, volcanic eruptions, and geothermal waters. Beside natural sources, anthropogenic activities like mining and smelting processes, metallurgical operations and coal combustion, wood preservatives, and use of herbicides and pesticides play an important role in arsenic contamination in the environment (McArthur et al. 2001; Bhattacharyya et al. 2003; Kossoff and Hudson-Edwards 2012; Sailo and Mahanta 2014).

Cadmium finds its way into the environment through natural as well as anthropogenic sources. Natural sources include volcanic eruption, oceanic spray, and forest fires. The key anthropogenic sources of Cd contamination to the environment are coal mining, metal-ore refining, fossil fuel combustion, cadmium-containing pigments, phosphate fertilizers, alloys, electronic compounds, detergents, and rechargeable batteries. Chromium enters the environment naturally by weathering of rocks, oil and coal burning, volcanic eruption, soil and sediments, and anthropogenically enters from sewage, fertilizers, combustion of fossil fuels, manufacturing of plastics, chromate production, and metal electroplating (Mishra and Bharagava 2016). Paper, pulp, and rubber manufacturing, and use of chromium in the leather and tannery industries (Mohan et al. 2006; Saxena et al. 2017), are further anthropogenic sources. A large amount of wastewater is discharged from industries due to extensive use of chromium compounds which contain toxic chromium species.

Lead is a common, abundant, and toxic heavy metal. In the earth's crust, 0.1% by weight lead occurs in rocks and soil. Lead is also found naturally in some plants. Exposure to lead is associated with more than 900 industries, and it accumulates in soil as dust. Anthropogenic sources include mining, smelting, refining, and battery manufacturing (Karrari et al. 2012). Applications in fertilizers and pesticides, sewage sludge application as irrigation from polluted water bodies, effluent discharge from industries to rivers, and coal-based thermal power plants contribute to soil lead pollution (Jalali and Khanlari 2008).

Three forms of mercury exist in nature-elemental (metallic), inorganic salts, and organomercurial compounds. An average of 0.08 mg $kg^{-1}$ Hg is present in the earth's crust and enters the environment from ocean evaporation, weathering of rocks and soils, forest fires, and volcanic gases. Anaerobic bacteria convert soil-bound Hg into dimethyl mercury. Plants take up Pb and release it as mercury vapour during transpiration. Mercury reaches the environment through anthropogenic activities like agriculture, burning of fossil fuels, municipal and industrial wastewater discharge, paper manufacturing, extensive use of the metal in industries, mining, and electrical appliances (Chen et al. 2012).

Naturally, nickel is found in soil and volcanic rock and enters into rivers and other water bodies through leaching of minerals and weathering of rocks and soils. Zinc is an essential element that occurs naturally in soil (approximately 70 mg $kg^{-1}$ in crustal rocks) (Davies and Jones 1998). Zn enters the environment through industrial applications in mining and metallurgical processing of zinc ores and coal burning. Anthropogenic origin of these heavy metals in the soil tends to be more mobile and bioavailable than natural ones (Kuo et al. 1983).Table 4.1 shows the major heavy metals and their different sources.

## 4.3 Toxicity of Heavy Metals

The most abundant and commonly found heavy metals at contaminated sites are Pb, Hg, As, Cr, Cd, Zn, and Cu (USEPA 1996). Heavy metals are oxidized to carbon (IV) oxide in soil by the action of microorganisms. However, most of the heavy metals are not degraded by chemical or microbial action and their concentrations persist in soil for long periods of time (Adriano 2003). Metal toxicity affects many aquatic bodies as well as water quality criteria, and these problems are exacerbated because metals can transport along with sediments, enter into the environment, and finally bioaccumulate in the food chain. The presence of heavy metals in the soil, air, and water pose a serious threat to humans through skin absorption, contact with contaminated soil, direct ingestion of contaminated foods through bioaccumulation up the food chain, and drinking of contaminated ground water. Heavy metals may come in contact with humans through residential activities (e.g. fertilizers and pesticides),

**Table 4.1** Different sources of heavy metals

| Metal | Various sources |
|---|---|
| Aluminium | Cooking utensils, city water supplies, ore smelting plants, anti-perspirants, cosmetics, automotive exhausts, pesticides |
| Arsenic | Fuel burning, mining, thermal power plants, smelting operations, combustion of coal, production of iron and steel, tobacco smoke, metal foundries, specialty glass products, ore smelting plants, weed killers, pesticides, fungicides, wood preservatives |
| Cadmium | Waste batteries, e-waste, welding, electroplating, ceramic glazes/enamels, cigarette smoke, food (from cadmium-contaminated soil), tap and well water, pesticides, fungicides, sewage, paints sludge, mines, incinerations and fuel combustion, power and smelting plants, seafood |
| Chromium | Mines, mineral sources, industrial coolants, chromium salts used in manufacturing, leather tanning, sewage, and fertilizers |
| Copper | Copper cookware, jewellery, dental alloys, water from copper pipe, mining, electroplating, smelting operations, fungicides, pesticides |
| Lead | Fuel combustion, lead in industrial processes, gasoline, smelting operations, solid waste combustion, metal plating and finishing operations, used in ceramics and dishware, PVC mini-blinds, coal-based thermal power plants, fertilizers, pesticides |
| Mercury | Solid waste combustion, mining, smelting, thermal power plants, fluorescent lamps, batteries, thermometers, barometers, dental amalgam fillings, cosmetics, pesticides, insecticides, fungicides, paints, laxatives, fish, shellfish, tap and well water, thermostats, vaccines |
| Zinc | Metal plating, electroplating, smelting, refineries, plumbing, brass manufacture |
| Nickel | Thermal power plants, electroplating, smelting, batteries |

industrial processes (e.g., manufacturing), pharmaceuticals, and agriculture. Heavy metals enter into human body through ingestion and absorption, and it becomes injurious to human health when their rate of accumulation outnumbers the rate of discharge.

### *4.3.1 Toxicity of Heavy Metals to Animals*

Generally, small amounts of heavy metals are necessary for good health, but prolonged exposure and higher concentrations become toxic or detrimental to human health. Toxicity of heavy metals can cause chronic, degenerative conditions, reduced energy levels, damage vital organs, and lower blood quality. Prolonged exposure of heavy metals can cause physical, muscular, and neurological degenerative problems, cancer-like diseases, and eventually death (Jarup 2003). Examples of health effects of heavy metals and route of entry to the human body are summarized in Table 4.2.

**Table 4.2** Route of entry and the health effects of important heavy metals

| Metal | Route of entry | Health effects | Symptoms/disease | References |
|---|---|---|---|---|
| Pb | Inhalation of dust particles or aerosols, consumption of Pb-contaminated food, water, and dermal contact | Higher concentration of the metal may damage the foetus and central nervous system. Lead toxicity harms kidneys, liver, haemoglobin synthesis, endocrine system, reproductive system, irreversible brain damage | Irritability, headache, poor attention span, dullness and memory loss, nausea, insomnia, anorexia, anaemia | NSC (2009); Wuana and Okieimen (2011) |
| Cr | Inhalation, through the skin | Higher concentrations cause skin ulceration, damage the kidney and affect the central nervous system. Occupational and environmental exposure to Cr (VI)-containing compounds cause asthma, allergy, and cancer | Vomiting, persisting diarrhoea, anaemia, irritation, and ulcers in the stomach | Goyer (2001) |
| As | Inhalation, ingestion, dermal contact, and the parenteral route to some extent | Carcinogen to skin, lung, bladder, kidney, gastrointestinal damage, birth defects, diarrhoea, severe vomiting, and death | Weakness, skin lesions, sloughing, fever, hypovolemic shock, anorexia, gastrointestinal pain, peripheral vascular disease, pulmonary disease, diabetes mellitus, hypertension, cardiovascular disease, hematemesis, haemolysis, jaundice, proteinuria, haematuria | Tchounwou et al. (1999); Smith et al. (2000) |
| Zn | Ingestion | Increased cholesterol levels and anaemia | Abdominal pain, lack of muscular coordination, nausea, and vomiting in children | Wuana and Okieimen (2011) |
| Cd | Inhalation and ingestion | Renal dysfunction, lung cancer, increase blood pressure, oxidative stress, and enzymatic systems of cells | Headaches, weakness, chills, vomiting, nausea, diarrhoea, pulmonary oedema. “Itai-Itai” disease caused by Cd toxicity results in multiple fractures arising from osteomalacia | Murata et al. (1970) |

(continued)

**Table 4.2** (continued)

| Metal | Route of entry | Health effects | Symptoms/disease | References |
|---|---|---|---|---|
| Cu | Ingestion of Cu-contaminated food | Irritation of stomach and intestine, anaemia, kidney and liver damage, central nervous system damage, and depression | Vomiting, diarrhoea, and loss of strength | Wuana and Okieimen (2011) |
| Hg | Absorption through skin, inhalation, ingestion, consumption of contaminated aquatic animals | Methyl mercury is highly toxic and harmful to the central nervous system and causes adverse neurological and behavioural changes; DNA damage, brain dysfunction, reproductive effects (i.e. birth defects, miscarriages, and sperm damage) | Symptoms: itching, rashes, redness, skin peeling to the nose and soles of the feet, tension, headaches, sleeplessness, vomiting, irritability, fatigue. Methyl mercury toxicity causes Minamata disease in Japan | Jan et al. (2015) |

### *4.3.2 Toxicity of Heavy Metals to Plants*

Plants require trace amounts of certain heavy metals like Zn, Cu, Fe, Mo, Mn, Ni, and Co for their growth, but higher level of these metals may be harmful and toxic to the plants. Forest canopy trees capture the air pollutants that get accumulated on their leaf surface. Concentrations of metals higher than optimum levels adversely affect the plant both directly and indirectly. Some examples of negative impacts caused by heavy metals on plants are inhibition of cytoplasmic enzymes and plant growth by chromium; photosynthesis inhibition by Cu and Hg; inhibition of seed germination and decrease of lipid content by Cd; structural damage of cells due to oxidative stress; decrease of plant growth and reduction of chlorophyll content by Pb; and reduction of seed germination by Ni (Gardea-Torresdey et al. 2005). Indirect effects include affecting microbial activities that affect plant growth and replacement of important nutrients at the cation exchange sites of the plants (Taiz and Zeiger 2002).

In plants, cadmium, lead, and nickel are highly toxic at comparatively lower levels. Cadmium is bio-persistent and has a high residence period. Plants grown in soil contaminated with Cd gradually take up the metal, which accumulate in their tissue. Noticeable symptoms like growth inhibition, chlorosis, root tip browning, and finally death commonly follows due to Cd toxicity. Cd adversely affects enzymatic activities, creating oxidative stress leading to nutritional deficiency in plants (Irfan et al. 2013). High concentration of Cd within plants reduced the nitrate reductase enzyme activity in shoots, resulting in the reduction of nitrate absorption and its transport from roots to shoots.

Most of the lead absorbed from the soil remains in plant roots. Lead toxicity affects plant morphology, growth, and photosynthetic processes and causes abnormal morphology and lignification of cortical parenchyma (Paivoke 1983). High Pb concentrations induce reactive oxygen species (ROS) and damage the lipid membrane, which causes adverse impacts to chlorophyll and inhibits photosynthetic processes and ultimately affects the overall plant development (Najeeb et al. 2014). Production of ROS may also damage nucleic acids and proteins and cause structural damage to cells. Nickel is essential in small doses; however, high concentrations of Ni in soil cause some physiological alterations in plants, chlorosis, and necrosis symptoms (Rahman et al. 2005). Ni toxicity changes water balance, reduces enzyme activity, and decreases chlorophyll content and stomatal conductance in pigeon pea (*Cajanus cajan*).

Chromium toxicity includes inhibition of seed germination, leaf chlorosis, root growth reduction, and reduction in plant biomass. Chlorosis and necrosis symptoms are found due to chromium toxicity in plants (Ghani 2011). Oxidative stress caused by chromium toxicity leads to degradation of photosynthetic pigments in plants. Higher concentration of zinc in soil inhibits plant metabolic activities, resulting in leaf senescence, and retarded root and shoot growth. Toxicity of zinc causes chlorosis in younger leaves, and after prolonged exposure, it extends to older leaves (Ebbs and Kochian 1997).

## 4.4 Remediation of Heavy Metals from Water and Wastewater

The majority of heavy metals cause adverse impacts on living organisms at very low doses and their remediation from the contaminated sites is a challenging but pivotal task. Several methods have been investigated for detoxification of heavy metals present in aquatic ecosystems. Physicochemical methods include adsorption, filtration, chemical precipitation/coagulation, membrane separation, and solvent extraction (Table 4.3).

Phytoremediation has been popularized for the remediation of toxic substances from the contaminated environment while being environmentally friendly and cost-effective (Bauddh and Singh 2012; Bauddh et al. 2016; Bharagava et al. 2017a, b). Although it is a slow process, it provides several value-added benefits such as carbon and nutrient sequestration, biofuel production, and aesthetic values (Bauddh et al. 2015; Chakravarty et al. 2017; Kumar et al. 2017).

Application of algae for the removal of water contaminants is currently observed in wastewater ecosystems. This process of remediation is termed ‘phycoremediation’ in which micro- or macro-algae are used for the decontamination of wastewater. Phycoremediation is used for the removal of excess nutrients, heavy metals, pesticides, dyes, and metal nanoparticles (Ettajani et al. 2001; Pawlik-Skowronska 2003; Chakravarty et al. 2015; Hultberg et al. 2016; Delgadillo-Mirquez et al. 2016;

**Table 4.3** Conventional methods for the removal of heavy metals from contaminated water and wastewater (Volesky 2001; O'Connell et al. 2008; Monteiro et al. 2012)

| Name of method | Mechanism | Advantage | Disadvantage |
|---|---|---|---|
| Adsorption | Removal by binding of contaminants on the surface of adsorbents | Applicable to wide range of contaminants | Adsorbent dependent |
| Membrane filtration | Removing contaminants from water by membrane filtration | No waste as by-product | High cost and difficult maintenance |
| Ion exchange | Exchange of water contaminants as ions by non-hazardous/beneficial ions | High efficiency | High cost and difficult maintenance |
| Reverse osmosis | Applied high pressure to contaminants from high concentration towards low concentration | High efficiency | High cost |
| Electrochemical treatment | Applied electricity to remove the dissolved contaminants | No chemicals required for the process | Expensive |

Babu et al. 2018). The wastewater is rich in nutrients that are required for algal growth and development like nitrogen, phosphorous, potassium, and other chemicals (Becker 1994; Dominic et al. 2009; Renuka et al. 2013, 2015; Whitton et al. 2015). The cultivation of algae in wastewater also reduces the cost of biodiesel production that requires significant quantities of water, nutrients, light, and energy for equipment (Gupta et al. 2015, 2016). Several researchers have studied using wastewater as a medium for the cultivation of algae for the production of biofuels (Chevalier et al. 2000; Xin et al. 2010; Park et al. 2010; Zhou et al. 2011; Gupta et al. 2015, 2016), which further reduces the cost of phycoremediation.

## 4.5 Phycoremediation

Wastewater bears diverse groups of inorganic and organic chemicals that can serve as suitable growth conditions for both micro and macroalgae (Olguí 2003; Cai et al. 2013; Gupta et al. 2016). The use of algae for the decontamination of majority of wastewater has been proved to be very effective by several researchers (Cai et al. 2013; Gupta et al. 2016). Algae-based removal of heavy metals works on two major processes: first is through metabolism-dependent uptake and second is biosorption (i.e. adsorption of metals on the algal cells) (Matagi et al. 1998; Afkar et al. 2010). A number of algal species like *Scenedesmus acutus, Chlorella vulgaris*, *Lemna minor, Nostoc muscorum*, *Phormidium ambiguum*, *Pseudochlorococcum typicum*, *Scenedesmus quadricauda,* and *Spirogyra hyaline* have been found to have excellent abilities to extract toxic metals from water and wastewater (Travieso et al. 1999; Peña-Castro et al. 2004; Kumar and Oommen 2012; Shanab et al. 2012; Dixit and Singh 2014; Singh et al. 2016; Gupta et al. 2017; Samadani et al. 2018).

Samadani et al. (2018) studied the bioaccumulation of Cd in *Chlamydomonas reinhardtii* and an acid-tolerant strain CPCC 121 during 48 h at two different pH conditions. *C. reinhardtii* was found to bear a greater Cd uptake in comparison with the strain CPCC 121. Ajayan et al. (2015) used Scenedesmus sp. for the remediation of Cr, Cu, Pb, and Zn from tannery wastewater. *Scenedesmus* removed Cr by 81.2–96%, Cu by 73.2–98%, Pb by 75–98%, and Zn by 65–98%.

Ajayan et al. (2011) studied the accumulation of Cu, Co, Zn, and Pb by *Scenedesmus bijuga* and *Oscillatoria quadripunctulata* through cultivating them in sewage water and petrochemical effluent. They found that both the species accumulated significant amounts of all the studied heavy metals, and the rate of accumulation from sewage wastewater and petrochemical effluent was 37–50, 20.3–33.3, 34.6–100, and 32.1–100%, respectively, for *O. quadripunctulata* and 60–50, 29.6–66, 15.4–25 and 42.9–50%, respectively, for *S. bijuga*. In a study conducted by Singh et al. (2016), *Lemna minor*, an aquatic plant commonly known as Duckweed, was found to accumulate As up to 735 mg $kg^{-1}$ in its leaves. *Lemna minor* was cultivated in an aquatic area naturally contaminated by As. Kim et al. (2003) evaluated heavy metal (Cu, Cd, Cr, Zn, and Pb) accumulation potential of the brown macroalga *Sargassum horneri*. The metal accumulated was in the order of Zn > Cu > Cr > Pb > Cd. Chen and Pan (2005) examined applicability of the Cyanobacteria genus *Spirulina* for the removal of Pb from wastewater. Adsorption rate of *Spirulina* was found 74% of Pd and the maximum biosorption efficiency of *Spirulina* cells was 0.62 mg Pb per $10^5$ algal cells.

The microalga *Scenedesmus incrassatulus* was used to remove three heavy metals (Cu, Cd, and Cr) growing in wastewater (Peña-Castro et al. 2004). Cr and Cd were found to bear significant positive interaction which enhanced the removal of both metals. *S. incrassatulus* efficiently removed the metals by 25–78%. Travieso et al. (1999) studied the effects of Cd, Cr, and Zn on the growth of *Scenedesmus acutus* and *Chlorella vulgaris* and their metal accumulation capacity. Both microalgae had good metal tolerance capacity, allowing these algae to remove higher concentrations of these toxic metals. Maximum Cd, Zn, and Cr removal efficiency of *Chlorella vulgaris* was 38–78% and for *Scenedesmus acutus* was 31–91%. Three microalgae (*Pseudochlorococcum typicum, Phormidium ambiguum*, and *Scenedesmus quadricauda*) were studied for the removal of Hg, Pb, and Cd in aqueous solutions by Shanab et al. (2012). They found that Hg caused severe toxic effects even at low concentration. However, initial concentrations of two other metals (Pb and Cd) increased algal growth. The removal of Hg, Pb, and Cd by *P. typicum* was highest 97% for Hg, followed by 86% for Cd, and 70% for Pb in the first 30 min of contact time. Azizi et al. (2012) used Oscillatoria sp. for biosorption of Cd cultivated in artificial aqueous solution. They observed that this alga has good potential for biosorption of the metal, and various factors influence the biosorption rate.

Kumar and Oommen (2012) used dry biomass of *Spirogyra hyaline* for the removal of five metals Hg, Cd, Pb, As, and Co. The highest amount of metals such as As, Cd, and Hg were removed at lower metal concentrations (i.e. 40 mg $L^{-1}$); however, Co and Pb exhibited the highest removal at 80 mg $L^{-1}$. The metal adsorption by dry biomass was found in order of Hg > Pb > Cd > As > Co. In a study conducted by Rehman and Shakoori (2004), *Chlorella* sp. found to have substantial tolerance against Cd at the concentration of 10 mg/mL and Ni at the concentration of 12 mg $mL^{-1}$. Chlorella removed significant amounts of both the metals. The reduction of Cd after 28 days from solution was up to 96%, and in the reduction of Ni from the medium after 28 days was 94%. *Tetraselmis suecica* and *Skeletonema costatum* were found to be hyperaccumulators of Cd (Ettajani et al. 2001). Zhou et al. (2012) studied Zn and Cu removal potential by two marine algae *Chlorella pyrenoidosa* and *Scenedesmus obliquus*. Both the species were found to bear approximately 100% metal removal ability. The applications of algal species in the removal of various heavy metals are shown in Table 4.4.

## *4.5.1 Factors Influencing Phycoremediation*

Removal of toxic metals by using algae is a natural and cost-effective technique, and it depends on several factors like type and level of contaminants, algal species, algal biomass, temperature, pH, presence of nutrients, and other factors (Selatina et al. 2004; Murugesan et al. 2006; Zeraatkar et al. 2016; Samadani et al. 2018). Biological factors like interspecies competition and presence of microorganisms (bacteria, viruses, and fungi) influence the growth of the phycoremediator algal species and may create noticeable limiting parameters (Grobbelaar 2000; González-Fernández et al. 2011).

### 4.5.1.1 Effect of pH

Among various factors that can influence the biosorption of metal ions, pH of the solution is a pivotal parameter (Matheickal and Yu 1996; Selatina et al. 2004; Samadani et al. 2018). pH alters the properties of the growing medium, metal binding sites of the sorbents, as well as properties of the metal ions (Esposito et al. 2001; Selatina et al. 2004; Vijayaraghavan and Yun 2008; Monteiro et al. 2012). Several studies reported that metal removal rate is enhanced with increased pH, while decreased pH values reduce the metal sorption efficiency of algal species (Mehta and Gaur 2001; Chojnacka et al. 2005; Gupta et al. 2006; Doshi et al. 2007; Liping et al. 2008; Abdel-Aty et al. 2013; Li et al. 2017). Sheng et al. (2004) reported that optimum pH for highest biosorption for Pb and Cu is 5.0 and for Cd, Zn, and Ni is pH 5.5 for marine algae *Padina* sp., *Gracilaria* sp., *Sargassum* sp., and *Ulva* sp.

Many researchers have observed that increased pH (5.0–6.0) reduces electrostatic repulsions between the adsorbents' surface and metals, resulting in increased metal removal by the algae (Ibrahim 2011; Momcilovic et al. 2011; Hassan et al. 2014). Samadani et al. (2018) studied phycoremediation potential of two algal species (*Chlamydomonas reinhardtii* and CPCC 121, an acid-tolerant strain) in two pH conditions (pH 4 and pH 7). At pH 7, Cd removal ability of *C. reinhardtii* was found

**Table 4.4** Application of several algal species for the removal of heavy metal

| Algal species | Metal(s) | Medium of cultivation | References |
|---|---|---|---|
| *Scenedesmus acutus* and *Chlorella vulgaris* | Cd, Zn, and Cr | Synthetic growth medium | Travieso et al. (1999) |
| *Nostoc rivularis* and *N. linckia* | Cd and Zn | Sewage water | El-Enany and Issa (2000) |
| *Sargassum horneri* | Cu, Cd, Cr, Zn, and Pb | Natural coast | Kim et al. (2003) |
| *Scenedesmus incrassatulus* | Cr, Cd, and Cu | Artificial wastewater | Peña-Castro et al. (2004) |
| *Chlorella* sp. | Cd and Ni | Synthetic growth medium | Rehman and Shakoori (2004) |
| *Anabaena subcylindrica* and *Nostoc muscorum* | Cu, Mn, Co, and Pb | Industrial wastewater and Sewage | El-Sheekh et al. (2003) |
| *Spirulina sps* | Pb | Synthetic growth medium | Chen and Pan (2005) |
| *Cladophora fascicularis* | Pb and Cu | Aqueous solution | Liping et al. (2008) |
| *Scenedesmus bijuga* and *Oscillatoria quadripunctulata* | Cu, Co, Pb, Zn | Sewage water | Ajayan et al. (2011) |
| *Caulerpa racemosa* and *Sargassum wightii* | Cr, Pb, and Cd | Aqueous solution | Tamilselvan et al. (2012) |
| *Oscillatoria* sp. | Cd | Artificial aqueous solution | Azizi et al. (2012) |
| *Spirogyra hyalina* | Cd, Hg, P, As, and Co | Artificial aqueous solution | Kumar and Oommen (2012) |
| *Ulva lactuca* | Cd | Artificial aqueous solution | Lupea et al. (2012) |
| *Pseudochlorococcum typicum, Phormidium ambiguum,* and *Scenedesmus quadricauda* | Hg, Pb, and Cd | Artificial aqueous solution | Shanab et al. (2012) |
| *Chlorella pyrenoidosa* and *Scenedesmus obliquus* | Zn and Cu | Artificial aqueous solution | Zhou et al. (2012) |
| *Nostoc muscorum* | Cd and Pb | Metal solution | Dixit and Singh (2014) |
| *Spirulina platensis* | Cd | Artificial aqueous solution | Al-Homaidan et al. (2015) |
| *Scenedesmus* sp. | Cr, CU, Pb, and Zn | Tannery wastewater | Ajayan et al. (2015) |
| *Lemna minor* | As | Natural contaminated area | Singh et al. (2016) |
| *Chlamydomonas reinhardtii* | Cd | Artificial aqueous solution | Samadani et al. (2018) |

to be limited due to its toxicity that was also dependent on exposure time and concentration of the metal in the growing medium. Rangsayatorn et al. (2004) reported insignificant removal of Cd by *Spirulina platensis* at pH 3.0; however, at higher pH up to 8.0, Cd adsorption increased substantially.

#### 4.5.1.2 Effect of Temperature

Temperature of the medium is also an important limiting factor for the removal of metal contaminants of the aquatic ecosystems. Temperature may alter the metal removal potential of algal species and the chemistry of heavy metals. Higher temperature increased metal-binding ability of algae by enhancing surface activity as well as kinetic energy of the contaminants (Skowronski 1986; Sag and Kutsal 2000; Mehta et al. 2002; Vijayaraghavan and Yun 2007). Few studies indicate that the sorption of metal ions reduced with increased temperature (Suhasini et al. 1999; Benquell and Benaissa 2002; Sari et al. 2007; Herrero et al. 2008).

#### 4.5.1.3 Effect of Contact Time

Heavy metal removal efficiency of algal biomass is influenced by contact time (Murugesan et al. 2006; Aroua et al. 2008; Wu et al. 2008; Saif et al. 2012). Ibrahim (2011) observed that *Ulva lactuca* and its activated carbon have maximum sorption capacity at a contact time of 60 min for Cd, Cr, Pb, and Cu ions. They also reported that after 60 min the adsorption rate was almost constant. Abdel-Aty et al. (2013) studied the impact of contact time on removal of Pb and Cd by *Anabaena sphaerica*. They found that the biosorption of the metals was faster in the initial 20 min, and after that it was gradually increased up to 60 and 90 min for Cd and Pb, respectively. Chen et al. (2008) reported a similar trend of Ni and Cu removal by *Undaria pinnatifida* and contact time.

#### 4.5.1.4 Effect of Biomass Concentration

In phycoremediation, biomass concentration has been found to be a strong metal biosorption-influencing factor (Nuhoglu et al. 2002; Gong et al. 2005; Karthikeyan et al. 2007; Sari and Tuzen 2008; Abdel-Aty et al. 2013). Increased biomass in the growing medium adversely influences the biosorption (Hamdy 2000; Nuhoglu et al. 2002; Gong et al. 2005). This is probably due to the aggregate's formation of the biosorbent at higher biomass that decreases the surface area of the biosorbents (Karthikeyan et al. 2007; Sari and Tuzen 2008). Abdel-Aty et al. (2013) found that the biosorption of Pb and Cd by *Anabaena sphaerica* was enhanced by increasing biosorbent and became constant at higher dosage from 0.1 g 100 $mL^{-1}$ for Pb and 0.2 g 100 $mL^{-1}$ for Cd. Al-Homaidan et al. (2015) reported that the biosorption efficiency of *Spirulina platensis* for Cd increases with higher biomass from 0.25 to 2 g; however, at 1.75 and 2 g doses, no substantial change occurred during the study. Solisio et al. (2008) observed that the dose of 2.0 g of *Spirulina platensis* is sufficient for the removal of Cd up to 98%.

#### 4.5.1.5 Effect of Metal Ion Concentration

The removal of metal ions basically depends on the concentration of metal present in the medium (Saleem and Bhatti 2011; Al-Homaidan et al. 2015). Generally, the biosorption of metal ions increases if the metal concentration in the medium increases, but this is up to a certain limit, and thereafter, the biosorption may be saturated or decreased (Aloysius et al. 1999; Saleem and Bhatti 2011; Lupea et al. 2012; Al-Homaidan et al. 2015). Biosorption of Cd by *Ulva lactuca* increased at metal concentration from 22.53 to 540.62 mg $L^{-1}$; however, Cd removal decreased from 80.78 to 42.43% at higher doses of the metal (Lupea et al. 2012). Ibrahim (2011) reported that the biosorption of Cu, Cr, Cd, and Pb by *Ulva lactuca* increased in the beginning and achieved maximum removal by 87.5% at 60 mg $L^{-1}$. After this dose of metal ions, the rate of adsorption was largely unchanged. Further increased metal level decreased the adsorption process.

## 4.6 Carbon Sequestration Potential of Algae

Climate change is a serious global issue. Some debate that it is caused by various natural internal or external processes, but the majority of credible scientists that the main driver of global climate change (GCC) is anthropogenic. $CO_2$ is the chief greenhouse gas (GHG), and its sequestration through algae is an important tool for mitigation of GHG (Eloka-Eboka and Inambao 2017). Although the global warming potential of $CO_2$ is lower than methane ($CH_3$), increased sources emitting $CO_2$ is what makes it the most serious GHG. The option of sequestering $CO_2$ naturally through biological means is attractive because plants naturally capture $CO_2$ through photosynthesis (Maraskolhe et al. 2012). Carbon capture along with bioenergy production (creating biofuel through algal oils) can play a pivotal role for $CO_2$ mitigation (Moreira and Pires 2016).

In aquatic ecosystems, both macro- and microalgae play a significant role in capturing carbon. The carbon is sequestered by these algal species in the form of bicarbonates ($HCO_3$) or $CO_2$. Carbon is the basic constituent of all organic molecules. Accumulated stored oils can be harvested to produce bioethanol, biodiesel, polyunsaturated fatty acids, and other bio-products (Spolaore et al. 2006; Milledge 2011; Razzak et al. 2013; Klinthong et al. 2015).

Different species of algae have different tolerance levels for $CO_2$. Ono and Cuello (2003) found that *Cyanidium caldarium* was the most $CO_2$-tolerant species they studied. However, several other species like *Scenedesmus* sp., *Chlorococcum* *littorale, Synechococcus elongatus,* and *Euglena gracilis* have also been found to have good tolerance levels (60–80%).

Creating a huge carbon sink would require large areas of aquatic habitat as well as large capital expense. The use of algae has been seen as an economical and feasible solution in this regard. Microalgae are more photosynthetically efficient than terrestrial plants because they have greater access to nutrients, water, and $CO_2$ and can thus more efficiently convert solar energy into biomass (Maraskolhe et al. 2012). It has been estimated that about 173 Tg C $year^{-1}$ of $CO_2$ could be globally sequestered by macroalgae. They can fix $CO_2$ from various sources like industrial exhaust, soluble carbonate salts, and other sources. Generally used microalgae include Chlorophyceae (green algae), Cyanophyceae (blue-green algae, which are actually photosynthetic bacteria), Bacillariophyceae (diatoms), and Chrysophyceae (golden algae).

The carbon sequestration potential of algae can be further enhanced by manipulating key enzymes through genetic engineering (Bajhaiy et al. 2017). The amount of $CO_2$ fixation plays an important role in affecting various metabolic processes of the cell including carbohydrate, lipid, and biomass synthesis (Wang et al. 2008). One of the advantages associated with capturing carbon in aquatic habitats is the efficiency to sequester $CO_2$ in the non-gaseous form of bicarbonates that fertilizes algal growth. At pH $\geq 7$ and temperature $<30$ °C, bicarbonate is the dominant form of $CO_2$ in water. Algae have active pumps for bicarbonate, and they have the ability to concentrate bicarbonates into their cell. This bicarbonate is then dehydrated, either by carbonic anhydrase or spontaneously, resulting in $CO_2$ trapped by the Calvin cycle. 1.6–2.0 g of $CO_2$ can be trapped per gram of algae biomass (Herzog and Golomb 2004).

Flue gas emitted from fossil fuel power plants has high emissions of $CO_2$ along with SOx and NOx gases. When this flue gas is injected into algal ponds, it increases algal biomass yield by almost threefold, but this requires a large amount of energy (Jeong et al. 2003). There are multiple factors that influence $CO_2$ sequestration, such as pH, temperature, oxides of sulphur (SOx), and oxides of nitrogen (NOx) in the flue gas, light, and other factors. It can be concluded that algae have a high carbon sequestration potential provided there are optimum conditions.

## 4.7 Bioenergy Production by Algae

Demand for energy has seen an unprecedented rise in the recent past, resulting in increased consumption of fossil fuel. There is still a sufficient supply of fossil fuel available economically, but using fossil fuel at this rapid rate is not safe for longer periods of time mainly due to rising GHG emissions. GHGs pose a severe impact on the environment through contributions to GCG. Therefore, there is a dire need to identify alternative sources of renewable energy that are economical as well as carbon neutral (Voloshin et al. 2016).

Biofuels are available for usage at a commercial scale such as bioethanol obtained from corn starch or sugarcane and biodiesel from oil crops, but crop-based biofuels raise critical ‘food versus fuel’ impacts on society (Demirbas 2009; Pittman et al. 2011). The advantages of using microalgae to sequester $CO_2$ and provide biofuel and other by-products is that they are not considered a ‘food’ crop for humans, they grow rapidly, and different species can be grown in saltwater, freshwater, and in polluted sewage and industrial water.

There are a number of advantages of using biodiesel produced from algae, including it does not have sulphur content; it is a ‘drop-in’ fuel (i.e. its chemical composition is quite similar to fossil fuel gasoline and can thus be used with little modification in internal combustion engines); and it generates less CO, SOx, NOx, and hydrocarbons emissions than combusting fossil fuels (Tokusoglu and Una 2003). Thus, biofuels obtained from cultivation of algae provides an alternative fuel that does not negatively affect agriculture. Algae like *Scenedesmus, Botryococcus,* and *Chlorella* have been tested for the production of biodiesel (Wang et al. 2013; Nascimento et al. 2013). Currently, researchers are focussing on maximizing the biodiesel yield from suitable species of algae, and much research and development is being done to increase the biodiesel production through cheaper and more effective technology. Biodiesel feedstock obtained from plants like Jatropha and Karanja needs to be pre-treated before production of biodiesel, which is not required for algae, which is yet another advantage (Delucchi 2003).

Wastewater proves to be an encouraging resource for the cultivation of microalgae (Chen et al. 2015). Combining cultivation of microalgae with treatment of wastewater (Fig. 4.2) can help in reducing $CO_2$ emissions, reduce nutrient pollution, and lowering the cost of biofuel production through not having to add fertilizer to enhance algal growth.

Some species of algae can accumulate high amount of lipids (Table 4.5) within their cells that may be exploited as feedstock for producing biodiesel, but intensive research is required to support this potential and optimize the systems of cultivation and harvesting of biomass (Simionato et al. 2013; Kumar et al. 2015). Production of biodiesel by lipid extracted from microalgae can be coupled with other energy processes that can make biodiesel a sustainable and economical product (Ansari et al. 2017).

Microalgal biotechnology shows a way forward as it can increase lipid and biomass productivity. Despite tremendous potential, traditional approaches are in dire need of improvement for increased lipid accumulation, so that they can be used for commercialization of biodiesel (Ravindran et al. 2017). However, production of biofuel has received a lot of attention currently, and it has the potential to replace the fossil fuels (Milano et al. 2016). Despite algae being considered as an alternative fuel, they are yet to attain techno-economic sustainability. Currently, the production of algal biofuel is costly (Lundquist et al. 2010). Biomass production faces a few technical issues with biomass production, processing of lipids, production of biofuel, and harvesting (Shriwastav and Gupta 2017).

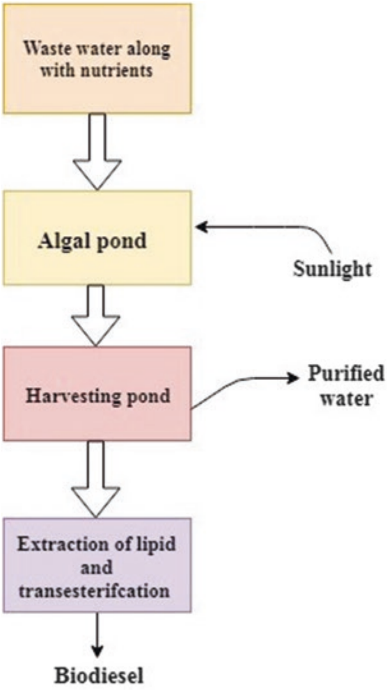

**Fig. 4.2** A flowchart illustrating the production of biodiesel from algae in wastewater systems

**Table 4.5** The lipid content of some microalgae (Gouveia and Oliveira 2009)

| Species | Lipids (% dry matter) |
|---|---|
| *Spirulina maxima* | 4–9 |
| *Chlorella minutissima* | 57 |
| *Dunaliella salina* | 14–20 |
| *Chlorella vulgaris* | 14–40/56 |
| *Chlorella emersonii* | 63 |
| *Chlorella sorokiniana* | 22 |
| *Dunaliella bioculata* | 8 |
| *Neochloris oleoabundans* | 3565 |
| *Scenedesmus obliquus* | 11–22/35–55 |
| *Chlorella protothecoides* | 23/55 |
| *Scenedesmus dimorphus* | 6–7/16–40 |

## 4.8 Conclusion

Toxic metal contamination of aquatic habitats is a severe environmental issue. Remediation of these contaminants through environmentally friendly technologies is a challenging task. Phycoremediation, especially of heavy metals, has emerged as a promising method that can synergistically remediate global environmental pollution through removing toxic metals along with other water contaminants like nutrients, dyes, pesticides, and metal nanoparticles; and coupling phycoremediation of contaminated water and wastewater with bioenergy production. Continued research advancements will hopefully remove the toxic heavy metals from contaminated water and wastewater by algae feasible and attractive for at commercial scale biodiesel production.

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