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Phycoremediation: Heavy Metals Green-Removal by Microalgae and Its Application in Biofuel Production

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Abstract

Heavy metals are non-degradable pollutants. Heavy metal accumulation in the environment increases along with the massive industrial activity and the growth of the human population. These pollutants are hazardous to various kinds of living things. The accumulation of heavy metals in the environment triggers bioaccumulation and biomagnification. Heavy metal exposure causes some health problems in humans. The conventional methods of heavy metals adsorption are less effective, expensive, and cause other problems. Phycoremediation using microalgae is a promising alternative method for absorbing heavy metals. Microalgal cells consist of various components such as lipids, polysaccharides, and proteins. Those compounds contain negative charges that will interact with positive charges on heavy metals. The bond between these different charges leads to the adsorption of heavy metals in the environment to the surface of microalgal cells. In this review, the authors discussed the potential use of microalgae to adsorb heavy metals polluting the environment. Phycoremediation is cheaper, easier, and safer for the environment. Besides, microalgal biomass can be utilized in biofuel production.

Keywords: Biofuel, Biosorption, Heavy Metals, Phycoremediation

1 Introduction

The growth of the human population has led to the increasing accumulation of pollutants in the environment. It also affects the decline in water quality caused by pollutant contamination in the aquatic environment. This problem is further exacerbated by high rate of urbanization and industrialization (1,2). Aquatic environment pollutants can be in the form of macro plastics waste, microplastic, (3,4), and heavy metals. Heavy metals are toxic and persistent non-biodegradable pollutants (2,5).

Heavy metals will be accumulated in food chains and have a prolonged effect on many living things. Heavy metals will also be changed from one compound into other compounds due to the characteristics of the heavy metals (6,7). Industrial wastewater containing heavy metals is a source of heavy metal pollution in the environment. The wastewater flows directly into waterways then will accumulate in the oceans. Heavy metals in the aquatic environment cause some detrimental effects on aquatic organisms like fish. It will indirectly cause health disorders in humans (8). Water quality is one of the essential factors supporting life for all organisms includes humans (9,10). Aquatic organisms are highly dependent on the water quality which is threatened by many pollutants polluting and decreasing the quality. Heavy metals pressurize the existence of various kinds of aquatic organisms. Contamination of aquatic organisms by heavy metals will also accumulate in humans through the food chains. Besides, contaminated water will also be consumed by humans (9,10). Heavy metals that exceed the threshold capacity in water are toxic to living things in the surrounding environment and tend to accumulate in organisms and move along with the food chain (11). The accumulation of heavy metals in the environment causes bioaccumulation. Bioaccumulation affects the food chains leading to health disorders for organisms exposed to these heavy metals (12). In the human body, heavy metals are transported by cells and bind to the protein, nucleic acids, lipids, and membranes. They will disrupt the function of cells (13). Also, the accumulation of heavy metals in waters poses a serious threat to human health (2).

Meanwhile, the conventional methods in adsorbing and eliminating heavy metals in the environment are expensive, less effective, and cause new environmental problems(14). Bioremediation is a promising method of environment recovery that is safe and friendly to the environment. This method involves various kinds of organisms as microalgae. Microalgae can be used as bio adsorbents to adsorb heavy metals(15). Bioremediation using microalgae is also known as phycoremediation (16). Heavy metals accumulated by microalgal cells will be involved in carbon metabolism in the cells. The produced carbon compounds can be utilized in the production of biofuels (17,18). Moreover, the presence of metals such as Fe increases the yield of biodiesel production in microalgae. It induces the production of carbon compounds in microalgal cells that are important in the production of microalgae-based biodiesel (18,19).

Recovery of the aquatic environment from all kinds of pollutants is necessary (20). In this review, the authors discussed

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the potential use of microalgae to adsorb heavy metals, especially in aquatic environments. The use of these microorganisms in adsorbing heavy metals can also be integrated with biomass production as feedstocks in biofuel production. Therefore, the phycoremediation of heavy metals by microalgae provides multiple benefits.

2 Heavy Metals and Their Hazardous Effects

Heavy metals are elements that have atomic weights ranging from 63.5 to 200.6. Heavy metals have a gravity of more than 5 (21,22). Metals with high concentrations of more than 5 µg categorized as heavy metals (13). In low concentrations, they are essential for living cells to carry out their metabolic activities. Metals such as vanadium, molybdenum, copper, iron, and zinc are needed by cells (21,22). Heavy metals are necessary elements in their cellular metabolism (15). However, if the concentration is exceeded, it will cause toxicity to the cells (21,22).

There are several sources of heavy metals. They are including chemical fertilizers and pesticides, household waste, industrial waste, and other human activities. The heavy metals that usually contaminate water environments are arsenic (As), mercury (Hg), zinc (Zn), nickel (Ni), Cadmium (Cd), copper (Cu), and lead (Pb) (15,23). Mining, metallurgy, and various chemical industries are some examples of activities producing heavy metals into the environment. These heavy metals are toxic to living cells includes humans. Heavy metals in the environment will enter the food chains that lead to bioaccumulation and biomagnification. Heavy metals are non-degradable substances and will remain for a long time (24).

Heavy metals are pollutants that are not biodegradable. Therefore they are hard to overcome in nature. One example of heavy metal is lead produced from various sources, such as the battery industry and the ceramic industry. Lead (Pb) is one of the heavy metals that pollute the aquatic environment (25). Pb is produced from the crystallization process from the air. The crystals are carried to the surface (water and land) through rain flow. This heavy metal also comes from the mineral deposits mediated by waves and rain, as well as from human activities as industrial activity waste (8).

Heavy metals have a long cycle and are difficult to eliminate from the environment. The accumulated heavy metals on the land will be carried to the aquatic environment. Their distribution depends on the physical and chemical properties of the soil containing these contaminants. These pollutants will directly reduce environmental quality. Heavy metals such as zinc, cadmium, lead, chrome, copper, and arsenic will be accumulated inside the body of various organisms includes plants and animals (26,27).

Along with industrial development, the amount of heavy metals polluting the environment, especially the aquatic environment, is also increasing. Industrial activities carry out the disposal of the waste directly to water bodies. It increases the accumulation of heavy metals in the aquatic environment. Among them, cadmium, mercury, and lead are the most dangerous heavy metals to humans. Also, heavy metals such as As, Br, and Cr are carcinogenic pollutants causing health disorders. In the aquatic environment, heavy metals will be accidentally consumed by various organisms as fish. They enter the food chains resulting in bioaccumulation and biomagnification (22,28).

Heavy metal pollution in aquatic environments leads to many problems. Humans who consume heavy metals accidentally will get several health problems. Accumulation of heavy metals in the body with high concentrations results in kidney problems, anemia, sclerosis, and nervous system disorders. The Accumulation of Cd can inhibit bone growth and cause nephritis, Cu causes Wilson's disease, and Zn causes metal fume fever (29). In addition, exposure to heavy metals in humans can cause infertility (20).

Heavy metal chromium (Cr) is one of the most toxic heavy metals (30). Cd is released from textile and photography industries apoptosis (31,32). It is an unessential substance for living cells due to its carcinogenic character. The accumulation of Cr inside the cell damages the chromatin structure and interferes with cell function (30). Also, it causes impaired cell wall integrity leading to kidneys, heart, and liver disorders. Moreover, Cr damages the mitochondria and endoplasmic reticulum releasing calcium in the cells that initiate cell apoptosis (31,32). It also replaces essential nutrients inside the cells (32).

Arsenic (As) is a lipid-soluble metal and can be absorbed through digestion, respiration, and direct skin contact. This element will be accumulated in the liver, kidneys, heart, and lungs. Excess arsenic causes poisoning with symptoms of vomiting accompanied by blood. Chronic poisoning can cause bleeding in the kidneys and skin cancer. Arsenic disturbs the oxidative phosphorylation process that damages mitochondria (13).

Fish and other aquatic resources are severely threatened by heavy metal pollution. Accumulation of heavy metals in aquatic products as fish will directly affect humans. In aquaculture, it must be ensured in advance that the water is free from heavy metal contamination (25). Research conducted by (11) reported that freshwater fish *Percocypris pingi* experienced 50% mortality after exposure to mercury with a concentration of 0.441 mg/L and 2.551 mg/L for cadmium within 24 hours (11). Also, aluminum 59.56 mg/L caused mortality *Catla catla*, 78.32 mg/L for *Labeo Rohita*, and 91.77 mg/L for *Cirrhina mrigala* within 24 hours (33).

3 Phycoremediation of Heavy Metals

The conventional methods to adsorb heavy metals from the environment are expensive and less effective. These methods also usually use other chemical compounds that cause further impacts on the environment. Nowadays, the development of methods for handling heavy metals is focused on biosorption using microorganisms. Microorganisms bind various metals because they have binding sites for metal ions in their cells (14). Bioremediation is a method of environmental recovery that is safe and friendly to the environment. This method involves several potential organisms like microalgae which can be used to absorb heavy metals. Microalgae can be utilized as alternative bio adsorbents in absorbing heavy metals (15).

Bioremediation using microalgae is also known as phycoremediation. Phycoremediation is a method of restoring a polluted environment that has several advantages compared to other conventional methods. Phycoremediation is easy to apply, cheap, safe, friendlier to the environment. This method has high effectiveness in adsorbing heavy metals. Additionally, the use of microalgae in phycoremediation will produce microalgae biomass. Biomass can be used in biorefinery activities for biodiesel production (11). Microalgae are abundant and large quantities in the aquatic environment. Mostly, they are not pathogenic and potential as agents for remediation of polluted aquatic environments. Microalgae have a rapid reproduction dan growth rate. Also, they are highly tolerant of various pollutants. Certain wastes can be utilized by microalgal cells as their energy and nutrition sources (34).

According to Bandurska (2007) cited in (27), within low concentrations, heavy metal as copper is needed for the cells of organisms. Copper plays an important role in cell metabolism and enzyme activity. The lack of this metal reduces the rate of enzyme activity. Copper also essential in the photosynthesis process as one of the constituent components of plastocyanin or chloroplast protein. They are useful in the electron transfer process (27). Some metals are essential for enzymes to carry out their activities and functions. Metals such as Pb act as a cofactor and activator for an enzyme. Some enzymes even contain certain metal ions on their active sites. The presence of these metals can activate enzyme function (8,35).

4 Phycoremediation of Heavy Metals by Microalgae

Phycoremediation using microalgae is one of the potential efforts to restore the environment from heavy metal pollution. Microalgae assimilate and bind heavy metals to their cells. The interaction between microalgal cells and heavy metals is through changes in ion permeability, chelation process, and active exclusion. Besides, these heavy metals will also be compartmentalized in microalgal cells (32,36).

The advantages of microalgae in the recovery of a polluted environment are easy to cultivate, relatively inexpensive, friendly to the environment, effective, and harmless (37). Heavy metals absorbed by microalgae can be extracted back from immobilized microalgal cells (38). The use of microalgae in bioremediation will also reduce the need for energy used and reduce the rate of carbon emissions and other pollutants into the environment (39). Some microalgal strains that have been reported as potential agents to absorb heavy metals are presented in Table 1.

One of the potential microalgae strains for heavy metal bioremediation is *Phacus* sp. It is a high tolerance strain to heavy metals. *Phacus* sp. Were able to absorb heavy metal Ni with a percentage of 75.17% with an initial concentration of 5 mg/L. The strain also reduced heavy metal Al by 19% within an initial concentration of 9.94 mg/L. Also, the percentage reduction in heavy metal Pb was 96.8% from an initial concentration of 1 mg/L (39).

Some genera of microalgae are capable of phycoremediation of the heavy metal cadmium. Cd replaces the function of Zn in the synthesis of carbonic anhydrase enzymes. The enzyme activity will produce hydrogen ions that will initiate cell division enhancing cell growth. Microalgae such as *Chlorella* sp. is also able to absorb and utilize this heavy metal and involves it in the metabolism of cell growth. *Chlorella* sp. also have other protective mechanisms against polluted environments including heavy metal contamination. This strain produces polyamine that will protect its cell and increase its tolerance to heavy metals (40).

Strains including *Ankistrodesmus* sp., *Chlorella* sp., and *Scenedesmus* sp. were reported to be able to adsorb Cd. The three microalgal strains were isolated from urban and rural water. It was known that *Scenedesmus* had the highest tolerance among the strains to the heavy metal cadmium. The LC_{50} value of cadmium against microalgae *Scenedesmus* sp. was 20.89 mg/L within 12 days. Meanwhile, the LC_{50} value of cadmium metal against *Chlorella* sp. was 8.00 mg/L. The LC_{50} for cadmium against *Ankistrodesmus* was 5.43 mg/L (32). The difference value of LC50 to microalgae influenced by several factors including light, temperature, cell density, and the type of media (41).

Furthermore, *Chlorella* sp. was reported by (42). In their study, 2 mL of microalgae were used and inoculated into a medium containing 10 mg of heavy metals and incubated for seven days at 20-24oC. The heavy metals included Cr, Cd, Cu, and Zn. Based on the obtained results, *Chlorella* sp. adsorbed as much as 33% Cr metal. Meanwhile, the adsorption percentages of Cu, Cd, and Cn were 29%, 15%, and 8%, respectively. The optimum salinity for metal adsorption was 34 and pH 7.

The removal percentage of heavy metal cadmium by microalgae *Chlorella* sp. was 8.07% with an initial concentration of 1 mg/L to 0.92 ± 0.082 mg/L. In a medium containing 7.0 mg/L cadmium, the removal percentage was 8.60% within seven days. Meanwhile, media containing 1 mg/L cadmium, *Scenedesmus* sp. removed 5.18% of the heavy metal from the growth medium. In media containing 7.0 mg/L cadmium, the microalgae removed 32.74% of the metal from the media. Based on the results, *Scenedesmus* sp. had higher tolerance and cadmium metal removal ability than *Chlorella* sp. (32). *Scenedesmus* sp. has cell walls consisting of several layers. It also releases extracellular polymeric substances containing anions that facilitate the accumulation of Cd into the microalgal cells. Besides, tolerance to heavy metals is also enhanced by the presence of protein which acts as a cation diffusion facilitator. This facilitator is essential in heavy metal homeostasis for microalgal cells (32,43).

Chlorella vulgaris reduced the concentration of heavy metal Pb with an initial concentration of 5 mg/L which could be reduced by 52% within 76 hours. Meanwhile, the rate of reduction of Cd metal by the microalgae after incubation for 76 hours was 51% with an initial concentration of 5 mg/L. The percentage reduction in heavy metals will decrease along with the increase of the concentration of heavy metals being tested. However, the number of heavy metals adsorbed will be higher at higher concentrations (15). *Chlorella sorokiniana* also has a high tolerance for heavy metals Cd and Pb (44).

Microalgae *Chlorella vulgaris* incubated in a medium containing walne fertilizer as a nutrient source and given Pb with a concentration of 5 mg/L absorbed the heavy metal as much as 0.5305 mg/L for seven days of incubation. This microalgal cell wall is composed of cellulose. Cellulose is essential in the heavy metal adsorption process. Cellulose has a polar OH functional group which will be effective in binding other polar substances in the environment. With the OH group, Pb will be bound to the cell wall of *C. vulgaris*. Meanwhile, the heavy metal will be processed through a detoxification mechanism by the microalgae cells to form a less toxic compound (8). While *C. vulgaris* incubated in a medium containing Pb, the first 3 hours was the phase of adaptation of the microalgae cells to the conditions of the medium containing Pb. The exponential phase occurred from the first to the seventh day (8). The cell wall *of Chlorella vulgaris* contains polysaccharides that contain alginate. Alginate is part of the polysaccharide that will bind to heavy metals. Metal ions such as Pb will be bound by alginate according to the following reaction (8,51).

$$3NaAlg + Pb3 \rightarrow Pb(Alg)_3 + 3Na$$

Table 1: Some Microalgal Strains Potential as Heavy Metals Bioadsorbents

Microalgae	Heavy Metals	Initial Concentrations	% Removal	Media	Incubation Time	Ref.
Botryococcus sp.	Zn	116. 67±3.51 ppb	45.20±0.59%	Domestic Wastewater	18 days	(45)
	Cd	0.34±0.04 ppb	56.97±3.54%	Domestic Wastewater	18 days	(45)
	Fe	302.19±3.02 ppb	45.00±0.86%	Domestic Wastewater	18 days	(45)
	Mn	55.17±0.76 ppb	86.18±1.15%	Domestic Wastewater	18 days	(45)
	Zn	141±3.00 ppb	56.95 ±2.70%	Food Processing Wastewater	18 days	(45)
	Cd	0.18±0.002 ppb	46.41±3.61%	Food Processing Wastewater	18 days	(45)
	Fe	455.33±2.53 ppb	34.11±1.56%	Food Processing Wastewater	18 days	(45)
	Mn	68.3±1.75 ppb	11.96±0.72%	Food Processing Wastewater	18 days	(45)
Chlorella sp.	Cd	7 mg/mL	8.60%	3N + V BBM	7 days	(32)
	Cd	10 mg	15%	Walne Media	7 days	(42)
	Cr	10 mg	33%	Walne Media	7 days	(42)
	Zn	10 mg	8%	Walne Media	7 days	(42)
	Cu	10 mg	29%	Walne Media	7 days	(42)
Chlorella vulgaris	Pb	5 mg/L	52%	Sea Water Containing Walne Fertilizer	76 days	(46)
	Cu	5 mg/L	51%	Sea Water Containing Walne Fertilizer	76 Days	(46)
Dunaliella sp.	Cd	30 mg/L	> 80%	Zarrouk Broth Medium	40 Hours	(24)
	Pb	30 mg/L	> 80%	Zarrouk Broth Medium	40 hours	(24)
	Hg	30 mg/L	> 80%	Zarrouk Broth Medium	40 Hours	(24)
	Pb	50 μg/L	96%	Culture Medium	6 days	(47)
Nannochloropsis sp.	Pb	0.9 ppm	11.46%	Sea Water And Walne Media	1 day	(48)
Pseudochlorococcu m typicum	Hg	10 μg/mL	98.17%	Bold's Basal Medium	24 Hours	(22)
	Cd	10 μg/mL	75.59%	Bold's Basal Medium	24 Hours	(22)
	Pb	10 μg/mL	61.76%	Bold's Basal Medium	24 Hours	(22)
Phacus sp.	Al	9.94 mg/L	64.28%	BG11	7 Days	(39)
	Ni	5 mg/L	66.67%	BG11	7 Days	(39)
	Pb	1 mg/L	79.17%	BG11	7 Days	(39)
Scenedesmus sp.	Cd	7 mg/mL	32.74%	3N + V BBM	7 days	(32)
Scenedesmus incrassatulus	Cr	2.09 mg/L	52.7±2.0%.	Modified Bold's Basal Medium	13-16 days	(49)
	Cd	7.36 mg/L	24.1±3.1%,	Modified Bold's Basal Medium	13-16 days	(49)
	Cu	6.57 mg/L.	31.7±3.4%,	Modified Bold's Basal Medium	13-16 days	(49)
Skeletonema costatum	Pb	2 ppm	80,5±0,5%	XMU Media	1 days	(25)
	Pb(II)	1 ppm	80.50%	XMU media	1 day	(50)
	Cd(II)	0.5 ppm	80%	XMU media	5 days	(50)
Spirogyra sp.	Cu	5 mg/L	89.6%	Modified Bold's Basal Medium	7 days	(7)
	Cr	5 mg/L	98.23%,	Modified Bold's Basal Medium	7 days	(7)
Microalgae	Heavy Metals	Initial Concentrations	% Removal	Media	Incubation Time	Ref.
Spirogyra sp.	Mn	5 mg/L	99.6%	Modified Bold's Basal Medium	7 days	(7)
	Se	5 mg/L	98.16	Modified Bold's Basal Medium	7 days	(7)
Spirogyra maxima	Pb	508.2 mg/L	>90%	Culture Medium	30 days	(29)
	Mn	274.9 mg/L	76.84%	Culture Medium	30 days	(29)
Spirulina sp. (*Arthospira* sp.)	Pb	0.9 ppm	12.54%	Sea Water and Walne Media	1 day	(48)
	Cu	5 mg/L	81.2%.	Spirulina Medium	7 days	(7)
	Cr	5 mg/L	98.3%	Spirulina Medium	7 days	(7)
	Mn	5 mg/L	99.73%,	Spirulina Medium	7 days	(7)
	Fe	5 mg/L	98.93%	Spirulina Medium	7 days	(7)
	Se	5 mg/L	98.83%,	Spirulina Medium	7 days	(7)
	Zn	5 mg/L	79%	Spirulina Medium	7 days	(7)

While *C. vulgaris* incubated in a medium containing Pb, the first 3 hours was the phase of adaptation of the microalgae cells to the conditions of the medium containing Pb. The exponential phase occurred from the first to the seventh day (8). The cell wall *of Chlorella vulgaris* contains polysaccharides that contain alginate. Alginate is part of the polysaccharide that will bind to heavy metals. Metal ions such as Pb will be bound by alginate according to the following reaction (8,51).

$$3NaAlg + Pb3 \rightarrow Pb(Alg)_3 + 3Na$$

Microalgae *Spirogyra maxima* also reported as heavy metals bio adsorbent. The removal efficiency of lead (Pb) reached more than 90% from the initial concentration of 508.2 mg/L within 30 days. During the same incubation period, it was able to absorb the heavy metal manganese (Mn) of 76.84% from the initial concentration of 274.9 mg/L. Based on this adsorption, it was known that the pH of the medium was decreased. Additionally, manganese (Mn) is used in the synthesis of chlorophyll (29).

S. costatum was also able to adsorb lead (Pb) in the media with a concentration of 2 ppm with a cell density of 5 x 10^4 cells/mL. The heavy metal adsorption efficiency was 80.5±0.5% on the first day of incubation. The percentage of the adsorption rate of Pb by the microalgae had decreased with the increase in incubation time. The ability of *S.costatum* to adsorb Pb is supported by the protein content found in its cells. The protein content of the cell has a carboxyl (-COOH) functional group which is important in binding heavy metal ions (25).

Lead (Pb) can also be adsorbed by the microalgae *Nannochloropsis* sp. and *Spirulina* sp. (*Arthospira* sp.). Based on research conducted by (48), *Nannochloropsis* sp. reduced the Pb content in the growth medium by 11.46% from the initial concentration of 0.9 ppm within 1 day and exposed to light for 12 hours using a 40-watt fluorescent lamp. Under the same conditions, *Spirulina* sp. reduced the Pb concentration by 12.54% (48).

Nannochloropsis sp. contains some functional groups that will bind heavy metal ions (48). *Spirulina* sp. contains high protein content reaching 77% of its biomass. Proteins consist of carboxyl groups binding heavy metal ions. Polysaccharides also essential because polysaccharides' hydroxyl groups interact with heavy metal ions (48,52). Lipids and proteins are two necessary components in binding heavy metals. Also, *Nannochloropsis* sp. does not have a mucus layer, therefore the rate of heavy metals transportation into cells will be easier. Besides, *Spirulina* sp. has high adaptability and easy to grow (48).

Microalgae *Dunaliella* sp. is more tolerant to heavy metal lead (Pb) than *Chlorella* sp. *Dunaliella* sp. biomass was increasing at the maximum Pb concentration of 150 g/L. However, the concentration of 200 g/L inhibited this microalgal growth. Within six days of incubation, *Dunaliella* sp. adsorbed 96% of 50 μg/L Pb in the media. Meanwhile, within the same concentration and incubation period, Pb was absorbed by *Chlorella* sp. by 34% (47).

Scenedesmus obliquus is one of the Cd^{2+} absorbing microalgae which absorption rate is relatively fast. The use of dead biomass can also adsorb the metals. Biomass efficiency in adsorbing heavy metals dependent on the additional nutrients (53). In a concentration of 5-10 μg/mL, Pb^{2+} and Cd are useful in the synthesize of chlorophyll a and protein in *Scenedesmus* sp. and *Pseudochlorococcum* sp. Pb^{2+} was significantly affecting the photosynthetic components of the two microalgae. However, heavy metal Hg^{2+} even in low concentration still damaged microalgal cells (22).

Spirulina sp. at the lag phase was tested for its biosorption capabilities to several heavy metals. It was cultivated on Spirulina medium containing heavy metals and incubated for 7 days. The adsorption percentage of heavy metal Cu with the initial concentration of 5 mg/L was 81.2%. The percentage of Cr, Fe, Mn, Se, and Zn were 98.3%, 98.93%, 99.73%, Se 98.83%, and 79% respectively. Also, *Spirogyra* sp. incubated on the medium containing the heavy metals at a concentration of 5 mg/L for 7 days was able to absorb heavy metals including Cu, Cr, Mn, Fe, Se, and Zn. The adsorption percentage for Cr was 98.23%, Cu 89.6%, Fe 99.73%, Mn 99.6%, Se 98.16%, and Zn 81.53% (7).

Botryococcus sp. reduced the concentration of heavy metals Zn, Cd, Mn, and Fe in food processing wastewater. It was incubated in the waste for 18 days and analyzed for its percentage reduction. *Botryococcus* sp. reduced Zn by 56.95±2.70% with an initial concentration of 141±3.00 ppb and a cell density of 1 $x10^7$ cells/mL. Within the same cell density, it reduced Fe by 34.11±1.56% with an initial concentration of 455.33±2.53 ppb. Meanwhile, the removal efficiency of Cd was 46.41±3.61% with an initial concentration of 0.18±0.002 ppb. Also, Mn adsorbed by *Botryococcus* sp. by 11.96±0.72% within the initial concentration of 68.3±1.75 ppb (45).

Botryococcus sp. adsorbed Zinc (Zn), Cadmium (Cd), Manganese (Mn), and iron (Fe) contained in domestic waste. With a cell density of 1 $x10^7$ cells/mL, it reduced Zn by 45.20±0.59% from the initial concentration of 116. 67±3.51 ppb (parts per billion). The adsorption percentage of Fe carried out by this microalgae was 45.00±0.86% in the initial concentration of 302.19±3.02 ppb. Cd was adsorbed 56.97±3.54% from the initial concentration of 0.34±0.04 ppb, and Mn was adsorbed by 86.18 ± 1.15% from the initial concentration of 55.17±0.76 ppb. The incubation period was 18 days (45).

Furthermore, *Dunaliella* sp. is also a high tolerance strain to heavy metals. *Dunaliella* sp. adsorbed heavy metals in its growth media. The heavy metals included Cd, Pb, and Hg. After being incubated for 40 hours, it was able to adsorb each metal more than 80% with an initial concentration of 30 mg/L. Additionally, the adsorption rate of heavy metals is categorized into two phases, fast adsorption, and slow absorption phase. The fast adsorption phase is the binding of heavy metals from the environment to the surface of microalgal cells. While the slow absorption rate occurs through translocation of heavy metals that had adsorbed on the surface of the cells into the microalgal cells (24).

Pseudochlorococcum typicum is another heavy metal tolerant microalgae. This strain adsorbed metal ions Hg^{2+}, Cd^{2+}, and Pb^{2+}. Within 24 hours, it adsorbed 98.17% of the Hg^{2+} with an initial concentration of 10 μg/mL. The percentage of Cd^{2+} adsorption was 75.59% with the same initial concentration, and the adsorption of Pb^{2+} was 61.76%. The adsorption increased with the increase of exposure time or contact with the metals. The concentration of 5 μg/mL of those metal ions was known to enhance the protein content and chlorophyll-a concentration. As for the microalgal cells exposed to the three heavy metals, they experienced changes compared to the normal cells. The cell surface and ultrastructures inclusions and cell organelles undergo some changes. After exposure to these metal ions, carbohydrate accumulation occurred around the microalgae pyrenoids of *Pseudochlorococcum typicum* (22).

Moreover, the absorption rate by *Phacus* sp. was higher and more optimum in media containing the mixed heavy metals (Al, Ni, and Pb) than in the medium that consists of single heavy metal. The adsorption rates of Ni, Al, and Pb were 66.67%, 64.28%, and 79.17%. Al-Pb expanded the surface of microalgal cells that triggered the efficiency of metal adsorption. The addition of heavy metal Pb to the medium with a concentration of 1 mg/L enhanced the growth rate of microalgal cells by 85.07% (39).

Scenedesmus incrassatulus was also reported as heavy metal (Cr, Cd, and Cu) bio adsorbent. It was cultivated in modified bold's basal medium and incubated for 13 to 16 days within a continuous system. The medium contained heavy metal Cd of 7.36 mg/L, Cr 2.09 mg/L, and Cu 6.57 mg/L. The adsorption of Cd was 24.1±3.1%, Cu 31.7±3.4%, and Cr 52.7±2.0%. This high adsorption rate was triggered by the use of the basal medium that enhanced the growth rate of microalgal biomass. Also, the removal rate of heavy metals in a continuous system was better than in the batch system. Meanwhile, mixing Cd, Cr, and Cu metals in the same medium increased the adsorption rate of Cu and Cd (49).

Dried biomass of *Spirogyra* sp. as much as 2.5 g was able to adsorb the heavy metal chromium in an initial concentration of

100 mg/L. In this concentration, it adsorbed as much as 13,236 mg/L or 13,236% within 1 hour contact time. The dried biomass also adsorbed copper as much as 59.82% from the initial concentration of 59.82% (54). Besides, *Scenedesmus quadicauda* and *Neochloris pseudoalveaolaris* were able to adsorb heavy metals Co, Cr, Pb, Cd, Ni, and Mn. Both types of microalgae cells are safe and easy to apply as heavy metal bio absorbents (55).

Research on the potential of microalgae to adsorb heavy metals is still focused on blue-green algae and green algae. Meanwhile, the report on the potential for diatoms to absorb heavy metals is still limited yet diatoms are abundant and easy to find in aquatic environments. Diatoms consisting of *Pseudostaurosira brevistriata* and *Staurosira construens* were effective in adsorbing silver (Ag). The adsorption of silver was related to the rate of photosynthesis of these cells. The diatoms also removed Pb with a maximum concentration of 30 ppm. These diatoms removed Cr and Cs contained in their growth media (56).

Skeletonema costatum was also reported for its ability to adsorb heavy metals cadmium(II) and Pb(II). The adsorption percentage of Pb (II) was 80.50% with a cell density of 5000 cells/mL that was incubated on the first day with a metal concentration of 1 ppm. The absorption of Cd(II) was 80% with an initial concentration of 0.5 ppm and incubated for 5 days at a cell density of 15,000 cells/mL. This permeability was inseparable from the presence of functional groups that provide bonds to heavy metals (50).

Skeletonema costatum was reported by (25). In their study, it was isolated from the Brackish Water Aquaculture Centers in Situbondo, Indonesia. The diatom was incubated in a medium containing different concentrations of lead (Pb) for 5 days. It was known that the optimum growth of *S.costatum* in media containing lead was on the second and third days with a cell density of 5000 cells/mL and 10000 cells/mL. The optimum growth rate in a medium containing 0.5 ppm and 2 ppm lead was within a cell density of 15000 cells/mL. It could be concluded that the denser the microalgal cells were, the more optimum their growth will be in the medium exposed to lead waste.

5 Mechanism of Heavy Metals Phycoremediation by Microalgae

There are two types of heavy metals biosorption, based on cell metabolism and based on the location of the heavy metals inside the cells and the cell surfaces. The absorptions of heavy metals that are influenced by cellular metabolism are dependent on cellular metabolism and independent of cellular metabolism. The metabolic dependence biosorption occurs through transport between cell membranes and precipitation. The absorption of heavy metals that do not depend on the metabolic activity occurs through precipitation, complexation, ion exchange, and physical absorption. The absorption of heavy metals based on their location is divided into adsorption or precipitation by the cell surface, extracellular adsorption, and intracellular absorption. Intracellular absorption occurs through transport between cell membranes. Cell surface adsorption occurs through ion exchange, complex formation, physical adsorption, and precipitation. Meanwhile, extracellular adsorption occurs through precipitation (57).

The adsorption of heavy metals by microalgae through active mechanisms occurs in waste containing heavy metals. Meanwhile, the passive adsorption of heavy metals can be applied using dried microalgal cells. These cells consist of charges that will bind to the charges found in heavy metal ions (18,58). The effectiveness of passive adsorption of heavy metals is highly dependent on the active site and properties of the microalgae. Those properties are the composition, size, and surface area of microalgal cells. Apart from that, the size and charge of the metal ions also affect the adsorption effectiveness. Also, the adsorption rate will be determined by environmental factors (18).

Heavy metals adsorption is involving cellulose contained in microalgal cell walls. Cellulose binds heavy metals from the environment. Cellulose contains functional groups such as hydroxyl that is essential in the adsorbing or binding of heavy metals (25,59). The functional groups bind metals based on the interaction between positive and negative charges on the cell walls and heavy metals. Functional groups found on the surface of the cell wall are negatively charged which will bind the positive charge on metal ions. The adsorption of heavy metals is also carried out by replacing the metals present in the microalgal cell walls (25). The positively charged heavy metal ions will bind to hydroxyl, carboxyl, carbonyl, and amino acid groups (37,60).

The functional groups present in organic compounds in microalgal cells are essential in absorbing metal ions from the environment. The charge contained in metal ions and functional groups will attract each other forming a bond. One example of the binding of a functional group to a metal ion is the sulfate functional group that binds to the metal ion Pb (lead). It is described in the following reaction (48):

$$\underset{\text{(Sulfate)}}{SO_4^{2-}} + \underset{\text{(Lead)}}{Pb^{2+}} \rightarrow \underset{\text{(Lead(II) sulfate)}}{Pb(SO_4)}$$

Metal ions will replace the monovalent and divalent ions found on the surface of the microalgal cell walls. This binding reduces the concentration of metal ions in the environment. However, not all metal ions will bind to the cell walls. Some metal ions will also bind with other metal ions that form a salt and be precipitated (46). Two valence metal ions will replace the divalent and monovalent ions present in the microalgal cell walls (15). The cell walls of *Chlorella* sp. are composed of cellulose, hemicellulose, glycoproteins, and pectins. These compounds have functional groups consisting of thiol and carboxylate groups which are important in binding heavy metal ions (37).

After the heavy metal ions are adsorbed and bound to the surface of the microalgal cells, the next process is the active uptake stage. This process occurs with the transfer of heavy metals to the microalgal cell organelles which leads to the bioaccumulation inside the microalgal cell organelles. The ions can be involved in the metabolic reactions of the cells. The heavy metal accumulation will correspond to the exposure time. The longer the exposure time, the more the accumulation of heavy metals (46).

The organic compound contained in microalgal cells such as chlorophyll is essential in binding heavy metals to form complex bonds. These bonds stabilize the heavy metals lead to heavy metal accumulation in the cells. Microalgae are also capable of producing heavy metal chelating agents enhancing them to absorb and accumulate heavy metals. *Chlorella* sp. is one of the microalgae that is capable of producing heavy metal chelating agents. The chelating compound consists of glutamate, glycine, and cystine that will chelate heavy metals in the cells (15,61).

There is a correlation between heavy metal exposure and protein synthesis in microalgal cells. The produced protein is in the form of phytochelatin that is important in the tolerance mechanism of microalgae to heavy metals (22). The phytochelatin produced by microalgae includes metallothionein class III (MtIII). It is essential in the detoxification of heavy metals (62). The MtIII biosynthesis can be initiated in the presence of heavy metals such as Cd^{2+},Au^{2+}, Cu^{2+}, Pb^{2+}, Ag^{2+}, Bi^{2+}, and Hg^{2+} (25,63).

Phytocelatin is a compound produced by microalgae that will chelate heavy metals. Phytocelatin contains sulfhydryl groups (-SH) binding heavy metal ions. The bound heavy metals will be accumulated in the vacuole and undergone a series of enzymatic reactions (8,35). According to Lehninger *et al*. (1993), phytocelatin is produced from glutathione which is induced when microalgae are present in an environment contaminated with heavy metal as Pb. Phytocelatin that is synthesized from tripeptide derivatives will react with heavy metals and translocate the heavy metal into the vacuole of microalgal cells (8).

Chlamydomonas reinhardtii exposed to several heavy metals (nickel, lead, and cadmium) expressed the gene encoding glutathione peroxidase enzyme. This enzyme is also essential in the tolerance of microalgal cells to heavy metals. Besides, the activity of the pyrroline-5-carboxylate synthetase and catalase enzymes also increased. It was known that nickel caused overexpression of the enzyme glutathione peroxidase, cadmium caused overexpression of glutathione peroxidase during 128 hours, but decreased after 168 hours (64).

Meanwhile, the further fate of the absorbed heavy metal by microalgae is the broken down and secreted or accumulated and metabolized. It depends on the chemical potential of the heavy metal compounds and their concentration. Hydrophilic heavy metals such as Cd, Hg, Pb, Co, and Cu will be more easily excreted than lipophilic compounds. However, the constant rate of heavy metal removal is lower than its adsorption rate. Thus, the heavy metals can still be strongly storage in the microalgae cell organelles (46).

6 Factors Affecting Biosorption of Heavy Metals by Microalgae

The absorption efficiency depends on the type of microalgae and the retention process. The type of microalgae is influenced by the size of the particles and the growth rate. The retention process includes the concentration of heavy metals, pH, contact time, and temperature (27,65).

One of the factors that determine the percentage of heavy metal absorption by microalgae is cell density. In low density, the absorption rate of heavy metals will be slow. As for maintaining and increasing the number of cells, it is necessary to add certain nutrients. The concentration of heavy metals also determines the absorption rate and percentage of heavy metal's adsorption. The higher the heavy metal concentration, the higher the absorption of heavy metals (15). Meanwhile, in high concentrations, heavy metals can reduce the content of chlorophyll a, chlorophyll b, and carotenoids in microalgae cells. Even. Zinc with a concentration of 100 mg/dm3 inhibits the growth process of *C.vulgaris* microalgae (27).

Microalgal species also determine the efficiency of heavy metal absorption. Each microalgal species has different cell characteristics. They have different cell walls and cell components. The composition and types of proteins and polysaccharides are also different. Thus, the affinity of heavy metals to each cell will be different (7). However, certain microalgae cannot live optimally in an environment contaminated with heavy metals. Therefore, the absorption rate of heavy metals takes a longer time due to some limitations on phycoremediation using certain living microalgal cells (18).

pH level also affects the absorption rate of heavy metals in microalgae. Heavy metals will more easily interact and form complexes with organic compounds found in microalgal cells at high pH. In high pH level, the redox potential will be lower which spurs the formation of complexes between heavy metals and organic compounds. It will also form chelates that are easy to dissolve (15). Increasing pH will cause various sides of the microalgae cells to deprotonate. The deprotonation occurred in acetamide chitin, phosphate, polysaccharide, amino acid, carboxyl group, and hydroxyl groups. Deprotonation of these components causes the binding of heavy metals to them will be higher (18).

The absorption of heavy metals such as Cd increases the pH due to the accumulation of H^+ ions. Increasing the pH will increase the absorption of heavy metals. The absorption of Cd by *Chlorella pyrenidosa* is more optimal at pH 7. When the pH is too high, the metal ions will bind with hydroxides to form metal hydroxides. If the environmental pH is too low, the formation of metal complexes with microalgae cell walls will be impeded by the accumulation of abundant H+ which will bind the cell walls of the microalgae (46).

Another factor affecting the percentage of heavy metal biosorption in microalgae is contact time. The longer the contact time between microalgae and heavy metals, the higher the absorption percentage of the heavy metals. The determination of the absorption percentage of microalgae is also influenced by the method (15). and nutrients. The addition of sufficient nutrients enhances microalgae growth. If the number of microalgae cells is low, the metal absorption rate will also be slow (46).

Immobilization of microalgal cells protects their cells and increases the efficiency of heavy metal absorption (66). *Spirogyra* dried biomass was more effective in absorbing heavy metal copper by 78% (67). Some components that can be used in immobilizing microalgae cells are chitosan, chitin, carrageenan, alginates, and cellulose. Alginate is the most frequently used symbolic component of microalgae cells due to its compatibility with microalgae cells. Alginate is also easy to apply (66,68).

The dried microalgae cells were reported to have better adsorption effectiveness of heavy metals than using living cells directly. The dead cells will adsorb heavy metals from the environment without rejection funds from the microalgal cells while living cells will not absorb exceed the concentration of heavy metal due to their toxicity to living cells (18,69).

Also, a consortium composed of *C.vulgaris* and *Enterobacter* sp. MN17 increased the absorption percentage of heavy metals compared to a single algae species. The mutualism symbiosis between the two microorganisms increased the removal rate of various heavy metals like Cr, Cd, Cu, and Pb, and also reduced COD. It initiated the absorption of heavy metals in the medium (70).

7 Biofuel Production

Phycoremediation using microalgae can be integrated with microalgal biomass production activities. Phycoremediation will not only reduce pollutants from the environment but can also produce biomass as biorefinery stock. Microalgae contain relatively high lipid and protein contents that are potential for the production of various components in biorefinery activities such as biofuel production (16). Microalgal biomass is a promising source of biofuel production materials. Microalgae can be grown outside their natural environment and do not require a large cultivation area. The growth rate of microalgae is also relatively rapid. The biomass produced from these microalgae can be processed through hydrothermal liquefaction to produce biofuels (18,71).

Microalgae are not only used for heavy metal remediation but the biomass can also be used as raw material for biodiesel production (42,72). Microalgae are the third biomass generation that is essential in biofuel production (73). Microalgae contain high lipid in their biomass. The lipid content of *C.vulgaris* was 25% with lipid productivity of 3.75 mg/lipid/day (74). *Spirulina* sp. which was incubated in salt pressure produced 25.53% lipid content (75).

Heavy metals that have been accumulated by microalgal cells will be involved in carbon compounds produced by these microalgae. These compounds can be processed in the production of biofuels. Thus, the heavy metals will not pollute the environment (17,18). The presence of metals such as Fe increases the yield of biodiesel in microalgae. It means that Fe induces the production of carbon compounds needed in microalgae-based biodiesel production (18,19).

Pb concentration affects the production and accumulation of lipids in the microalgal cells of *Scenedesmus* sp. at low concentrations, Pb has no effect on lipid production in this strain. At concentrations of 0.5 and 1 mg/L, Pb increased the lipid concentration. At this concentration, the accumulation of lipids within its cells reached more than 30%. However, in higher concentrations, the lipid concentration decreased. Therefore, it can be seen that heavy metals in suitable concentrations can increase lipid production in microalgal cells that are important in biofuel production (76). Moreover, Heavy metal Pb^{2+} with a concentration of 5 μg/mL to 10 μg/mL is known to induce the production of chlorophyll a and protein content in two types of microalgae consisting of *Scenedesmus* and *Pseudochlorococcum*. Additionally, in *Phormidium*, this concentration can also enhance the production of chlorophyll (22).

The production of biofuels has various advantages compared to fossil-based energy. Biofuel is renewable energy for the future (77). Biofuel production will use carbon sources from the atmosphere that have been processed by microalgae cells through the photosynthesis process. Thus, the use of biomass in biofuel production will not use carbon sources derived from fossils that can damage the environment. The use of biomass in producing biofuels is also renewable. Biofuels are also more environmentally friendly and do not cause problems like fossil fuels. Besides, biofuel production will also open up job opportunities for the communities (78). Furthermore, several techniques can be used in the pre-treatment of lignocellulosic biomass-based biofuel production. The techniques used include biological, mechanical, physical, and chemical techniques (77).

8 Conclusion and Future Perspective

Although the use of microalgae to absorb heavy metals has many advantages, there are also some limitations. The accumulated metal in the microalgal cells must also be considered to prevent biomagnification. Besides, the use of living microalgal cells directly is less optimal. The strength of the micro-body cell mechanism is relatively low, the excessive hydrostatic pressure will reduce the ability of microalgal cells to adsorb heavy metals (46).

One of the challenges is how to increase the ability of microalgae in absorbing heavy metals. The increase in the absorption ability of heavy metals will increase the efficiency of heavy metals removal. One of the efforts that must be done is by carrying out genetic engineering through the application of rDNA. Strain improvement is intended to trigger the overexpression of phytochelatin and increasing the synthesis of heavy metal-binding protein. Through the strain improvement, it is expected that the tolerance and absorption rate by microalgal cells will be higher and optimal (38).

In conclusion, the use of microalgae to absorb heavy metals in the environment is highly potential. The adsorption of heavy metals is urgently essential due to the presence of these heavy metals threatening various organisms. Bioaccumulation and biomagnification also threaten humans themselves. Phycoremediation has many advantages over other conventional methods in absorbing heavy metals. Phycoremediation is easier and cheaper to apply and does not cause other problems for the environment. This method can also be integrated with the production of biomass as feedstock in the biorefinery. Microalgae will grow and absorb these heavy metals, while the biomass can be used in the production of biofuels. Thus, the integrated process will provide more benefits.

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Ethical issue

Authors are aware of and comply with, best practices in publication ethics specifically concerning authorship (avoidance of guest authorship), dual submission, manipulation of figures, competing interests, and compliance with policies on research ethics. Authors adhere to publication requirements that submitted work is original and has not been published elsewhere in any language.

Competing interests

The authors declare that no conflict of interest would prejudice the impartiality of this scientific work.

Authors' contribution

All authors of this study have a complete contribution to data collection, data analyses, and manuscript writing.

References

1. Lalevic B, Raicevic V, Kikovis D, Jovanovic L, Surlan-Momirovic G, Jovic J, et al. Biodegradation of MTBE by Bacteria Isolated from Oil Hydrocarbons-Contaminated Environments. Int J Environt Res. 2012;6(1):81–6.
2. Rezania S, Ponraj M, Talaiekhozani A, Mohamad SE, Md Din MF, Taib SM, *et al.* Perspectives of phytoremediation using water hyacinth for removal of heavy metals, organic and inorganic pollutants in

wastewater. J Environ Manage. 2015;163:125–33. http://dx.doi.org/10.1016/j.jenvman.2015.08.018

3. Asiandu AP, Wahyudi A, Sari SW. A Review: Plastics Waste Biodegradation Using Plastics-Degrading Bacteria. J Environ Treat Tech. 2021;9(1):148–57.
4. Asiandu AP, Wahyudi A, Sari S. Biodegradation of plastics waste using fungi: A review. Curr Res Environ Appl Mycol. 2021;11(1):1–15.
5. Isarankura-na-ayudhya C, Tantimongcolwat T, Kongpanpee T, Prabkate P, Prachayasitikul V. Original article : Appropriate Technology for the Bioconversion of Water Hyacinth (*Eichhornia crassipes*) to Liquid Ethanol : Future Prospects for Community Strengthening and Sustainable Development. EXCLI J. 2007;6:167–76.
6. Volesky B. Detoxification of metal-bearing effluents: Biosorption for the next century. Hydrometallurgy. 2001;59(2–3):203–16.
7. Mane PC, Bhosle AB. Bioremoval of some metals by living algae *Spirogyra* sp. and *Spirullina* sp. from aqueous solution. Int J Environ Res. 2012;6(2):571–6.
8. Dewi ERS, Nuravivah R. Potential of Microalgae *Chlorella vulgaris* As Bioremediation Agents of Heavy Metal Pb (Lead) On Culture Media. E3S Web Conf. 2018;31:1–4.
9. Schreiber U, Müller JF, Haugg A, Gademann R. New type of dual-channel PAM chlorophyll fluorometer for highly sensitive water toxicity biotests. Photosynth Res. 2002;74(3):317–30.
10. Li M, Wan CY, Pan XJ, Zou Y, Chi SY, Chang JB. Comparative Study of Stress by Four Heavy Metals on *Chlamydomonas reinhardtii* and the Potential Application in BBE Algae Toximeter. Fresenius Environ Bull. 2013;22(5 A):1494–500.
11. Yuan D, Huang L, Zeng L, Liu S, He Z, Zhao M, et al. Acute Toxicity of Mercury Chloride (Hgcl2) and Cadmium Chloride (Cdcl2) on the Behavior of Freshwater Fish, *Percocypris Pingi*. Int J Aquac Fish Sci. 2017;3(3):066–70.
12. Mancera-Rodríguez NJ, Álvarez-León R. Current State of Knowledge of the Concentration of Mercury and Other Heavy Metals in Fresh Water Fish in Colombia. Acta Biológica Colomb. 2006;11(1):3–23.
13. Cope WQ, Leidy R., Hodgson E. Classes of Toxicants : Use Classes. In E. Hodgson. A Textoook of Modern Toxicology, 3rd ed. 3rd ed. Hodgson E, editor. New Jersey: A John Wiley & Sons, INC., Publication; 2004. 49–74 p.
14. Gurbuz F. Removal of toxic Hexavalent Chromium Ions from Aqueous Solution by a Natural Biomaterial: Batch and column adsorption. Adsorpt Sci Technol. 2009;27(8):745–59.
15. Purnamawati FS, Soeprobowati TR, Izzati M. Potensi *Chlorella vulgaris* Beijerinck dalam Remediasi Logam Berat Cd dan Pb Skala Laboratorium. Bioma Berk Ilm Biol. 2015;16(2):102–13.
16. Apandi N, Radin Mohamed RMS, Al-Gheethi A, Latiffi A, Arifin SNH, Gani P. Phycoremediation of Heavy Metals in Wet Market Wastewater. IOP Conf Ser Earth Environ Sci. 2018;140(1):1–6.
17. Raikova S, Smith-Baedorf H, Bransgrove R, Barlow O, Santomauro F, Wagner JL, et al. Assessing Hydrothermal Liquefaction for the Production of Bio-oil and Enhanced Metal Recovery from Microalgae Cultivated on Acid Mine Drainage. Fuel Process Technol. 2016;142:219–27.
18. Piccini M, Raikova S, Allen MJ, Chuck CJ. A Synergistic Use of Microalgae and Macroalgae for Heavy Metal Bioremediation and Bioenergy Production Through Hydrothermal Liquefaction. Sustain Energy Fuels. 2019;3(1):292–301.
19. Lu J, Watson J, Zeng J, Li H, Zhu Z, Wang M, et al. Biocrude Production and Heavy Metal Migration During Hydrothermal Liquefaction of Swine Manure. Process Saf Environ Prot. 2018;115:108–15. Available from: http://dx.doi.org/10.1016/j.psep.2017.11.001
20. Talaiekhozani A, Rezania S. Application of Photosynthetic Bacteria for Removal of Heavy Metals , Macro- Pollutants and Dye from Wastewater : A review. J Water Process Eng. 2017;19:312–21. http://dx.doi.org/10.1016/j.jwpe.2017.09.004
21. Srivastava NK, Majumder CB. Novel Biofiltration Methods for the Treatment of Heavy Metals from Industrial Wastewater. J Hazard Mater. 2008;151(1):1–8.
22. Shanab S, Essa A, Shalaby E. Bioremoval Capacity of Three Heavy Metals by Some Microalgae Species (Egyptian Isolates). Plant Signal Behav. 2012;7(3):392–9.
23. Akpor OB, Muchie M. Remediation of Heavy Metals in Drinking Water and Wastewater Treatment Systems: Processes and Applications. Int J Phys Sci. 2010;5(12):1807–17.
24. Imani S, Rezaei-Zarchi S, Hashemi M, Borna H, Javid A, Zand AM, et al. Hg, Cd and Pb heavy metal bioremediation by *Dunaliella* alga. J Med Plants Res. 2011;5(13):2275–780.
25. Soedarti T, Maryono LR, Hariyanto S. Bioremediation of Lead [Pb] Contaminated Sea Water by Marine Diaton *Skeletonema costatum*. Icsbe. 2016;263–70.
26. Węglarzy K. Metale ciężkie – źródła zanieczyszczeń i wpływ na środowisko[Heavy metals – the source of pollution and the effect on the environment]. Wiadomości Zootech. 2007;3:31–8.
27. Kondzior P, Butarewicz A. Effect of heavy metals (Cu and Zn) on the Content of Photosynthetic Pigments in the Cells of Algae *Chlorella vulgaris*. J Ecol Eng. 2018;19(3):18–28.
28. Wilde EW, Benemann JR. Bioremoval of heavy metals by the use of microalgae. Biotechnol Adv. 1993;11(4):781–812.
29. Nimisha P, Joseph S. Assessment of Phycoremediation Efficiency of *Spirogyra Maxima* by using Heavy Metals Manganese and Lead. Int J Environ Agric Biotechnol. 2020;5(3):616–20.
30. Widiatmono BR, Fajri Anugroho D, Arief T Munaf dan F. Pengaruh Kepadatan Mikroalga *Chlorella* sp. terhadap Bioremediasi Logam Krom Pada Limbah Cair Industri Penyamakan Kulit. Sumberd Alam dan Lingkung. 2018;5(3):6–14.
31. So KY, Oh SH. Cadmium-induced heme-oxygenase-1 Expression Plays Dual Roles in Autophagy and Apoptosis and is Regulated by both PKC-δ and PKB/Akt activation in NRK52E kidney cells. Toxicology. 2016;370:49–59.
http://dx.doi.org/10.1016/j.tox.2016.09.010
32. Dugue D, Montoya C, Botero L. Cadmium (Cd) tolerance evaluation of three strains of microalgae of the genus *Ankistrodesmus, Chlorella* and *Scenedesmus.* Rev Fac Ing Univ Antioquia. 2019;(92):60–9.
33. Azmat H, Javed M, Jabeen G. Acute Toxicity of Aluminium to the Fish (*Catla catla*, *Labeo rohita* and *Cirrhina mrigala*). Pak Vet J. 2012;32(1):85–7. http://www.pvj.com.pk/pdf-files/31_3/192-194.pdf
34. Olguín EJ. Phycoremediation: Key Sssues for Cost-Effective Nutrient Removal Processes. Biotechnol Adv. 2003;22:81–91.
35. Palar H. Pencemaran dan Toksilogi Logam Berat. Jakarta: Rineka Cipta; 2008.
36. Abdel-Raouf N, Al-Homaidan AA, Ibraheem IBM. Microalgae and wastewater Treatment. Saudi J Biol Sci. 2012;19(3):257–75.
http://dx.doi.org/10.1016/j.sjbs.2012.04.005
37. Hanifa L, Zulaika E. Potensi Sel *Chlorella* sp. Inaktif sebagai Biosorben Logam Berat Cd 2^+. J Sains dan Seni Pomits. 2014;3(2):33–5.
38. Halder PS. Bioremediation of heavy Metals Through Fresh Water Microalgae: A review. Sch Acad J Biosci. 2014;2(11):825–30.
39. Ahmad N, Mounsef JR, Tayeh JA, Lteif R. Bioremediation of Ni, Al and Pb by the Living Cells of a Resistant Strain of Microalga. Water Sci Technol. 2020;82(5):851–60.
40. Widiyani P, Dewi ERS. Penurunan Konsentrasi Logam Berat Kadmium (Cd) Dan Pertumbuhan Mikroalga Chlorella Vulgaris Pada Media Kultur. Bioma. 2014;3(2):17–26.
41. Ouyang HL, Kong XZ, He W, Qin N, He QS, Wang Y, et al. Effects of five Heavy Metals at sub-Lethal Concentrations on the Growth and Photosynthesis of *Chlorella vulgaris*. Chinese Sci Bull. 2012;57(25):3363–70.
42. Wetipo YS, Mangimbulude JC, Rondonowu FS. Potensi *Chlorella* sp. sebagai Agen Bioremediasi Logam Berat di Air. Pros

Semin Nas Pendidik X. 2013;10(1):115–9.

43. Das N, Bhattacharya S, Maiti MK. Enhanced Cadmium Accumulation and Tolerance in Transgenic Tobacco Overexpressing Rice Metal Tolerance Protein Gene OsMTP1 is promising for phytoremediation. Plant Physiol Biochem. 2016;105:297–309. http://dx.doi.org/10.1016/j.plaphy.2016.04.049

44. Carfagna S, Lanza N, Salbitani G, Basile A, Sorbo S, Vona V. Physiological and Morphological Responses of Lead or Cadmium exposed *Chlorella sorokiniana* 211-8K (Chlorophyceae). Springerplus. 2013;2(1):1–7.

45. Gani P, Mohamed Sunar N, Matias-Peralta H, Abdul Latiff AA, Parjo UK, Oyekanmi AA. Green Approach in the Bio-removal of Heavy Metals from wastewaters. MATEC Web Conf. 2017;103:1–8.

46. Purwaningrum P. Upaya Mengurangi Timbulan Sampah Plastik Di Lingkungan. Indones J Urban Environ Technol. 2016;8(2):141.

47. Muhaemin M. Toxicity and Bioaccumulation of Lead in *Chlorella* and *Dunaliella*. J Coast Dev. 2004;8(1):27–33.

48. Masithah ED, Rahardja BS, Hardianie TNOK. Comparative Study of Ability *Nannochloropsis* sp. and *Spirulina* sp. as Agent Bioremediation of Heavy Meal Plumbum (Pb). J Ilm Perikan dan Kelaut. 2011;3(2):167–73.

49. Peña-Castro JM, Martínez-Jerónimo F, Esparza-García F, Cañizares-Villanueva RO. Heavy Metals Removal by the Microalga *Scenedesmus incrassatulus* in Continuous Cultures. Bioresour Technol. 2004;94(2):219–22.

50. Soedarti T, Surtiningsih T, Hariyanto S, Kuncoro EP. Remediation of Pb(II) and Cd(II) in Sea Water by *Skeletonema costatum.* Sustinere J Environ Sustain. 2017;1(2):84–93.

51. Kurniawan JI, Aunurohim. Biosorpsi Logam $Zn2^+$ dan $Pb2^+$ oleh Mikroalga *Chlorella* sp. J Sains dan Seni ITS. 2014;3(1):1–6.

52. Babadzhanov AS, Abdusamatova N, Yusupova FM, Faizullaeva N, Mezhlumyan LG, Malikova MK. Chemical Composition Of *Spirulina platensis* Cultivated in Uzbekistan. Chem Nat Compd. 2004;40(3):276–9.

53. Monteiro CM, Castro PML, Malcata XX. Use of the Microalga *Scenedesmus obliquus* to Remove Cadmium Cations from Aqueous Solutions. World J Microbiol Biotechnol. 2009;25(9):1573–8.

54. Chatterjee A, Abraham J. Biosorption Capacity of Dried Spirogyra on Heavy Metals. Int J ChemTech Res. 2015;8(9):387–92.

55. Kizilkaya B, Türker G, Akgül R, Doğan F. Comparative Study of Biosorption of Heavy Metals Using Living Green Algae *Scenedesmus quadricauda* and *Neochloris pseudoalveolaris*: Equilibrium and Kinetics. J Dispers Sci Technol. 2012;33(3):410–9.

56. Kunugi M, Sekiguchi T, Onizawa H, Jimbo I. Utilization of Diatoms to Collect Metallic Ions. Proc Schl Eng Tokai Univ, Ser E. 2014;39:13–8.

57. Nichat AR, Shaffi SA, Kakaria VK. *Spirulina platensis* and *Chlorella vulgaris* Assisted Bioremediation of Heavy Metal Contaminated Aquatic Ecosystem. J Microb Biochem Technol. 2020;12(3):1–11.

58. Monteiro CM, Castro PML, Malcata FX. Metal uptake by microalgae: Underlying Mechanisms and Practical Applications. Biotechnol Prog. 2012;28(2):299–311.

59. Knauer K, Behra R, Sigg L. Adsorption and Uptake of Copper By the Green Alga *Scenedesmus subspicatus* (Chlorophyta). J Phycol. 1997;33:596–601.

60. Wang J, Chen C. Biosorbents for Heavy Metals Removal and Their Future. Biotechnol Adv [Internet]. 2009;27(2):195–226. http://dx.doi.org/10.1016/j.biotechadv.2008.11.002

61. Haryoto, Wibowo A. Kinetics Bioaccumulation Heavy Metal Cadmium by encironmental Fitoplankton *Chlorella* sp. tetirotial water sea. J Penelit Sains Teknol. 2004;5(2):89–103. //publikasiilmiah.ums.ac.id/bitstream/handle/11617/365/2. HARYOTO.pdf?sequence=1

62. Perales-Vela HV, Peña-Castro JM, Cañizares-Villanueva RO. Heavy Metal Detoxification in Eukaryotic Microalgae. Chemosphere. 2006;64(1):1–10.

63. Shaw AJ. Heavy Metal Tolerance in Plants: Evolutionary aspects. Bota Raton, Florida: CRC Press Inc.; 1989. 195–214 p.

64. Athanasiadis K, Abdelgawad H, Moubiana V, Asard H, Blust R. Heavy #ake from the Green Alga *Chlamydomonas reinhardtii* : Single Heavy Metal Uptake from the Green Alga *Chlamydomonas reinhardtii* : Single and Mix-Metal Exposure. 2018;(August 2017).

65. Bulgariu L, Gavrilescu M. Bioremediation of Heavy Metals by Microalgae [IN:] Handbook of Marine Microalgae Biotechnology Advances. Kim S-K. [ed.]. Elsevier. 2015. 457–469 p.

66. Petrovič A, Simonič M. Removal of Heavy Metal Ions From Drinking Water By Alginate-Immobilised *Chlorella sorokiniana*. Int J Environ Sci Technol. 2016;13(7):1761–80.

67. Kushwah A, Srivastav JK. Biosorption of Copper Ions by Green Algae *Spirogyra*. 2015;3(3):36–8.

68. Akhtar N, Saeed A, Iqbal M. *Chlorella sorokiniana* Immobilized On The Biomatrix Of Vegetable Sponge of *Luffa cylindrica*: A New System to Remove Cadmium from Contaminated Aqueous Medium. Bioresour Technol. 2003;88(2):163–5.

69. Davis TA, Volesky B, Mucci A. A Review of The Biochemistry of Heavy Metal Biosorption by Brown Algae. Water Res. 2003;37(18):4311–30.

70. Mubashar M, Naveed M, Mustafa A, Ashraf S, Baig KS, Alamri S, et al. Experimental Investigation of *Chlorella vulgaris* and *Enterobacter* sp. Mn17 for Decolorization and Removal of Heavy Metals from Textile Wastewater. Water. 2020;12(11):1–14.

71. Gollakota ARK, Kishore N, Gu S. A Review on Hydrothermal Liquefaction of Biomass. Renew Sustain Energy Rev. 2018;81:1378–92. Available from: http://dx.doi.org/10.1016/j.rser.2017.05.178

72. Mata TM, Martins AA, Caetano NS. Microalgae for Biodiesel Production and Other Applications: A review. Renew Sustain Energy Rev. 2010;14(1):217–32.

73. Alam F, Mobin S, Chowdhury H. Third generation biofuel from Algae. Procedia Eng. 2015;105:763–8. Available from: http://dx.doi.org/10.1016/j.proeng.2015.05.068

74. Aguoru CU, Okibe PO. Content and Composition of Lipid Produced By *Chlorella vulgaris* for Biodiesel Production. Adv Life Sci Technol. 2015;36:96–101.

75. Bhakar RN, Kumar R, Pabbi S. Total Lipids and Fatty Acid Profile of Different *Spirulina* Strains as Affected by Salinity and Incubation Time. Vegetos. 2013;26:148–54.

76. Pham TL, Dao TS, Bui HN, Pham TKN, Ngo TTH, Bui HM. Lipid Production Combined with Removal and Bioaccumulation of Pb By *Scenedesmus* sp. Green Alga. Polish J Environ Stud. 2020;29(2):1785–91.

77. Talaiekhozani A, Rezania S. A Critical Review on the Various Pretreatment Technologies of Lignocellulosic Materials. J Environemental Treat Tech. 2020;8(3):925–35.

78. Alaswad A, Dassisti M, Prescott T, Olabi AG. Technologies and developments of third generation Biofuel Production. Renew Sustain Energy Rev [Internet]. 2015;51:1446–60. Available from: http://dx.doi.org/10.1016/j.rser.2015.07.058