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REVIEW ARTICLE

The Potential Use of Algae as Biosorbents for Mercury Removal in the Indonesian Water Bodies

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ABSTRACT

Contamination of mercury in Indonesian water bodies has become a big concern for many people due to the harmful effects of this heavy metal when it enters the body. Mercury contamination may cause neurological disorders that lead to loss of senses; damage to the brain, the central nervous system, and the kidney; and can lead to birth defects. Human activities, such as coal-fired power plants (CFFPs) and artisanal and small-scale gold mining (ASGM) as well as other mining activities, are among the biggest contributors of mercury emission in Indonesia's water body. Biosorbents such as fungi, bacteria and algae can be utilized to alleviate this problem, with algae being the most reliable biosorbent due to its abundance in Indonesia, low-cost manufacturing, and high metal ion binding capacity. There are three varieties of algae that can be used as a mercury biosorbent: green algae (Chlorophyta), red algae (Rhodophyta), and brown algae (Phaeophyta). Different studies have said different conclusions for the most compatible mercury biosorbent, commonly between green and brown algae. Further research should be considered to know whether algae can be a reliable long-term alternative to water treatment compared to other research that uses the combination of chemical and physical treatments.

KEYWORDS

Indonesia; Water body; Algae; Mercury; Biosorbents

HIGHLIGHTS

- The presence of mercury in Indonesia's water bodies is causing significant concern due to its harmful effects on human health.
- Mercury contamination can lead to neurological disorders, causing a loss of senses, brain damage, and harm to the central nervous system and kidneys. Additionally, it poses a risk of birth defects.
- Among the biosorbents, algae stand out as the most reliable choice in Indonesia due to their abundance, low-cost manufacturing, and high capacity to bind with metal ions.
- Three types of algae, namely green algae (Chlorophyta), red algae (Rhodophyta), and brown algae (Phaeophyta), can serve as effective mercury biosorbents.
- Green algae exhibit the highest mercury absorption capacity, making them the most compatible biosorbent.

INTRODUCTION

The water body in Indonesia is contaminated with an abundance of heavy metals including cadmium (<0.30 ppm), lead (29.20 ppm), copper (38.61 ppm), zinc (98.33 ppm) and mercury (2.13 ppm) (Utomo & Effendi, 2022). The Kotopanjang dam in Pekanbaru, Riau, for example, was among the recent cases of heavily contaminated bodies of water with heavy metals, such as lead, cadmium, and zinc that are present and accumulated in the gills, kidney, and muscle tissues of the fish caught from this dam (Budijono & Hasbi, 2021). The groundwater area in Pati and Rembang, Central Java, were also found to be contaminated with mercury and arsenic caused by corrosion of metals from the buildings and ships, human activities, and industrial wastes (Rochaddi et al., 2021). Fish tissues collected from the Talawaan river in North Sulawesi were found to have a high concentration of mercury due to artisanal and small-scale gold mining (ASGM) activities in the nearby Tatelu village (Castilhos et al., 2006).

As the third largest mercury emitter in the world, Indonesia has to deal with the mercury contamination in the water bodies around the country, which has affected the life of over a million people who still rely much on an unsafe old-fashioned gold extraction and concentration method called whole-ore amalgamation for their ASGM purpose (Ismawati et al., 2013). The mercury contamination in freshwater bodies due to human activities will eventually end up in the ocean water. Although in theory, the main mercury emitter to the ocean is still mother nature, with erosion and geochemical cycle releasing more than five tons of mercury into the sea annually (Swain et al., 2007).

Mercury (Hg) is one of the most prevalent and toxic minerals found in Indonesian water bodies (Darmawan & Sriwahyuni, 2022). The Latin name hydrargyrum (Hg) means metal that looks like liquid silver due to the high malleability characteristic of mercury at standard temperature and pressure (Azevedo et al., 2012). It can be divided into three main groups that include elemental mercury (Hg), inorganic mercury (such as mercurous mercury (Hg^+) or mercuric mercury (Hg^{2+}) salts), and organic mercury or organometallic (when mercury forms a covalent bond with a carbon atom of an organic functional group such as a methyl, ethyl, or phenyl group) (Azevedo et al., 2012; Zulaikhah et al., 2020). Each mercury form has its own biological behavior, pharmacokinetics, and clinical significance, depending on their own chemical structure; but the most toxic form is methylmercury, an organic mercury (Bernhoft, 2012).

In general, liquid elemental mercury is poorly absorbed by the human body with very low health risk due to its bioavailability—which is less than 0.01% (Houston, 2007). However, elemental mercury has high solubility in lipids that makes it easily diffusible, and in a vapor form, elemental mercury can be inhaled easily, leading to absorption by the lungs (Azevedo et al., 2012). Once inside the human body, elemental mercury can then diffuse through the cell membranes as well as the blood-brain and placental barriers, reaching the target organs, the brain, and the kidney (Azevedo et al., 2012). The organs and red blood cells require a few minutes to convert the elemental mercury that has been absorbed into the inorganic mercuric form (Hg^{2+}). The inhaled elemental mercury vapor can possibly build up in the central nervous system causing damage to occur (Azevedo et al., 2012). During this time, a substantial amount of mercury vapor can cross the blood-brain barrier before it is oxidized and eventually accumulate in the brain. Through the olfactory route, elemental mercury can reach the brain from the nasal cavity (Park & Zheng, 2012).

An alternative to help tackle the presence of lethal methyl-mercury is through the use of biosorbents (Kumar et al., 2019). Biosorbent is a biological substance that has the ability to remove pollutants and is made from biological materials (Liu et al., 2021). There are three types of organisms that are commonly used as biosorbents: algae (the term algae in this review will be used to describe both micro and macroalgae), fungi, and bacteria. Fungi and bacteria are used as biosorbents due to their metal-binding capacity. Although both have the capability to be used as biosorbents, bacteria are known to have a complicated large-scale harvesting procedure that includes sorption, ion exchange, evaporation, chemical precipitation, reverse osmosis, and electrochemical treatment which involves high expense of operation (Mustapha & Halimoon, 2015). Fungi can result in incomplete metal removal due to its poor biosorption capacity as well as production of toxic side products (Dhankhar & Hooda, 2011; Lu et al., 2020; Wehrs et al., 2019). As a result, algae are preferred over bacteria and fungi due to their low cost for large-scale production, where they do not usually require any special treatment, are ecologically safer, and have a high metal ion binding capacity (Bilal et al., 2018; Bulgariu & Bulgariu, 2017).

Algae can be categorized into three types: red algae (Rhodophyta), brown algae (Phaeophyta), and green algae (Chlorophyta), wherein they are known as macroalgae (Beetul et al., 2016; Kariyawasam, 2016; Raven & Giordano, 2014; van Ginneken & de Vries, 2018). The unicellular forms of algae are known as microalgae, in which they are classified into four types: blue-green algae (Cyanophyta), dinoflagellates (Pyrrophyta), diatoms & golden-brown algae (Chrysopytha), microscopic green algae (Chlorophyta), and plastids (Cryptophyta) (Beetul et al., 2016). Each group of algae has its own characteristics that differentiate it from one to another. Based on their own characteristics, each group also has its own compatibility standard for a suitable use as a biosorbent (Chandler et al., 2016).

The unique characteristics in algae are related to the differences in pigmentation and cell wall (Bulgariu & Bulgariu, 2012; He & Chen, 2014). There are some algae that are incapable or do not perform well for biosorption such as Cryptophyta as it does not have a cell wall; Pyrrhophyta has "bare" or covered with cellulose "thecal" plates for protection; and Chrysopytha has naked or scaled cellulosic walls (Bold & Wynne, 1984; Lee, 1989). Cellulose makes up most of the cell wall in algae as this biomolecule helps provide structural support for algae. The functional groups on the surface of the algal cell wall (sulfate, hydroxyl, phosphate, imidazole, amino, amine) interact with the environment and can serve as binding sites where positively charged metal ions can interact with negatively charged groups on the cell wall surface (Michalak et al., 2013). In metal binding, covalent bonds between a metal ion and a functional group are formed by amino and carboxyl groups, whereas ionic bonds are formed by carboxyl and sulfate groups (Abbas et al., 2009). Among the functional groups, the carboxyl group plays an important role in biosorption, shown using an X-ray photoelectron spectroscopy (XPS) and Fourier-transform infrared spectroscopy (FTIR), demonstrating the interaction of the functional groups with the heavy metal in the adsorption process (Sheng et al., 2004).

The aim of this review is to provide a comparison between different algae in removing mercury from water bodies in Indonesia. This review covers the danger behind the presence of mercury in the water body, the effect that it has on its surroundings, and the utilization of algae as biosorbents. The characteristics of green, red, and brown algae are also discussed. The limitations of using algae as biosorbent are explained alongside ways to overcome these limitations and further research to help future prospects in this field.

MERCURY IN THE INDONESIAN WATER BODY

Natural and anthropogenic sources are the two main emitters of mercury on planet Earth, with mercuric sulfide or mercury (II) sulfide (HgS) found in insoluble red crystal cinnabar as the main natural form of mercury (National Research Council (US) Committee on the Toxicological Effects of Methylmercury, 2000; Rytuba, 2003). Mercury concentration in cinnabar varies greatly depending on the location of deposits due to its properties that do not allow geochemical blending between mercury with other elements found in the earth’s crust (Rytuba, 2003). The weather and volcanic activities can release mercury into the atmosphere from earth's sediment, and human activities, such as incineration and burning of fossil fuels, can also do the same (National Research Council (US) Committee on the Toxicological Effects of Methylmercury, 2000). Once released from the earth's crust, inorganic cation mercury (Hg^{+}/Hg^{2+}) can travel a long distance contaminate waterways due to run-off from either natural or anthropogenic sources, or from air deposition (National Research Council (US) Committee on the Toxicological Effects of Methyl Mercury, 2000).

Once in the water body, the inorganic mercury can be transformed into its most toxic organometallic cation form: methyl mercury ($[CH_3Hg]^+$ or in this review, MeHg is used). MeHg is produced by adding a methyl group to inorganic mercury through the metabolic activity of microorganisms (Park & Zheng, 2012). Several microorganisms that facilitate methylation in aqueous environments include sulfate-reducing bacteria like *Desulfovibrio desulfuricans*, methane-producing bacteria, and iron-reducing bacteria (Nogara et al., 2019). The occurrence of methylation can depend on the climate condition within the environment, which further affects the activity of microorganisms and oxygen levels (Cesário et al., 2017). Conditions with smaller oxygen concentrations promote the methylation process (Nogara et al., 2019).

Sulfate-reducing bacteria is one of the microorganisms that make a big contribution to the methylation process in anoxic estuarine sediment (Compeau & Bartha, 1985). The carbon-3 from amino acid serine in the bacteria gives a methyl group to tetrahydrofolate to produce 5,10-methylene-tetrahydrofolate (5,10-CH_2-THF). This reaction is catalyzed by hydroxy-methyl-transferase enzymes (Compeau & Bartha, 1985). A reduced ferredoxin then reduces 5,10-CH_2-THF into Me-THF (Berman et al., 1990). After that, the methyl group from Me-THF is transferred into Hg^{2+} by the cobalamin (B12) protein complex (Choi & Bartha, 1993). MeHg is then cleaved from the complex and goes out from the microorganism to the environment (Nogara et al., 2019).

MeHg in the water body can be transferred to more complex organisms, leading to bioaccumulation that brings bigger harm in higher species at the food chain (Nogara et al., 2019; Park & Zheng, 2012). Fish containing MeHg are often consumed by humans: a common route in MeHg exposure. The MeHg exposure from diet substantially varies in humans depending on the differences in frequency, amount, and type of fish consumed, as well as the MeHg concentration in the fish (Nogara et al., 2019). In some populations, such as the Canadian Inuits, MeHg exposure can be very high due to high consumption of contaminated mammals and birds (Calder et al., 2019).

Human activity has always been the main cause of mercury emissions to the environment (International Pollutants Elimination Network, 2018). The World Health Organization (WHO) estimated that 25% of the overall mercury emission to the atmosphere comes from anthropogenic sources (National Research Council (US) Committee on the Toxicological Effects of Methylmercury, 2000). The coal-fired power plants (CFPPs), poor industrial waste management, waste incineration, and gold or other metals mining are among the anthropogenic causes of mercury pollution (Sundseth et al., 2017).

The CFPPs burn coal that contains trapped mercury. The unique chemistry of mercury is that the metal is liquid at room temperature, but a moderate heat will evaporate it into mercury vapor. When coal is burned, mercury is released as a gas into the environment (Tchounwou et al., 2012). The amount of CFPPs in Indonesia showed a 10.4% annual growth between 2006 and 2015, resulting in an increase in mercury release from coal (IPEN, 2018). Research conducted by Basel and Stockholm Convention Regional Centre for South-East Asia (2017) stated that mercury emitted into the atmosphere from CFPPs in Indonesia was around 2.9 tonnes/year. However, despite the high mercury emission, Indonesia still has not made a policy regarding mercury emission limitations for CFPPs and other facilities that do coal burning. A high concentration of mercury pollution in the air will eventually be carried by rain into the water bodies, thus increasing the rate of mercury contamination in the aquatic systems (Zulaikhah et al., 2020).

Other than CFPPs, the artisanal and small-scale gold mining (ASGM) activity is another big contributor of mercury emissions in Indonesia (Futsaeter & Wilson, 2013). The ASGM industry often employs cheaper technology for gold searching and extraction, known as whole-ore amalgamation which is applied with non-standard safety and risk assessments (IPEN, 2018). Mercury waste from this process is then disposed into the environment as inorganic mercury, leading to pollution in waterways (Krisnayanti et al., 2012). The Indonesian government has since prohibited the use of mercury in mining activities, but due to the gold's high price, many people are still illegally practicing it by purchasing the mercury from the black market (Spiegel et al., 2018).

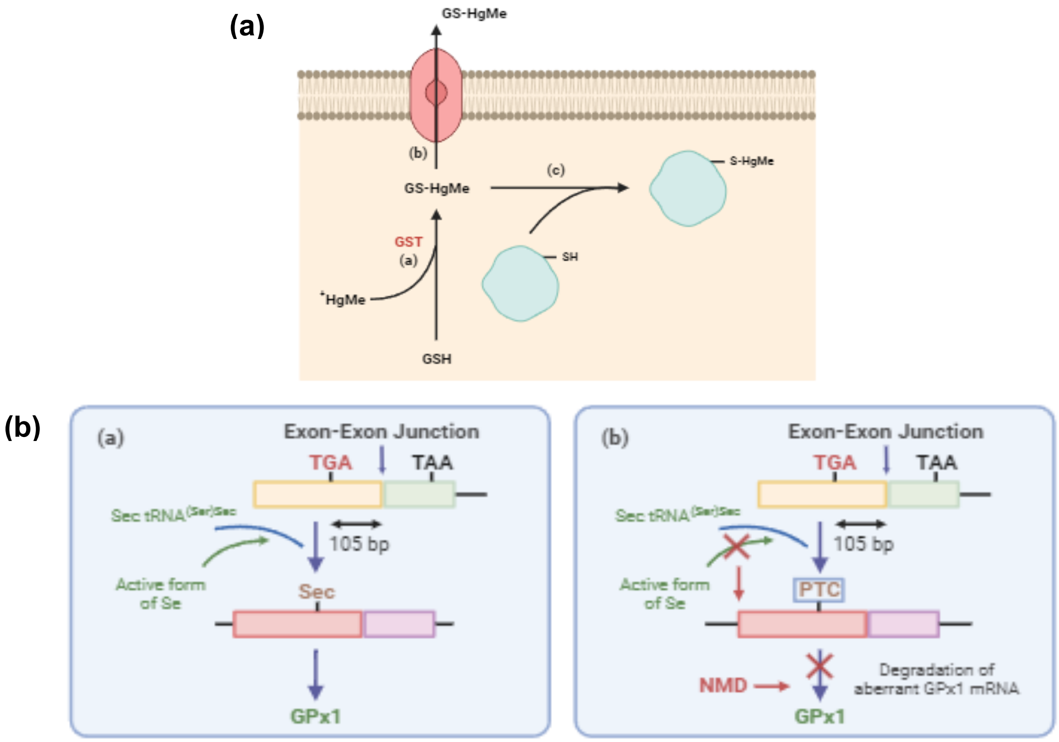
Episodic events such as storms and seasonal runoff carry the inorganic mercury from the ASGM sites into rivers, lakes, dams, or even the ocean (Schuster et al., 2008). Barkdull et al. (2019) tried to evaluate the mercury contamination in four different water streams near ASGM sites in Central Java. The results showed that in two of the four sites, the total dissolved mercury concentrations exceeded 12 ng/L, the standard safety criteria for aquatic organisms according to the United States Environmental Protection Agency (Barkdull et al., 2019). The highest mercury concentration was found in Jatiroto (see Figure 1), where it reached 4730 ng/L (Barkdull et al., 2019). The study by Barkdull et al. (2019) also revealed that the total mercury contamination in the water stream was not proportionally related to the scale of the mining site; Jatiroto was the second smallest mining site among the four, but it showed the highest concentration of mercury. Moreover, MeHg was found to be 20% of the total mercury forms collected from the stream, indicating high methylation occurrence in the area (Barkdull et al., 2019). The methylation may occur more easily in the water body in a tropical region like Indonesia due to anoxic floor sedimentation that is rich in dissolved organic carbon but low in oxygen concentration (Rothenberg et al., 2017).

An excessive amount of mercury is an environmental hazard for all organisms that inhabit the contaminated area (Briffa et al., 2020). Studies by Susanti et al. (2020) showed that mercury was found in duck meat samples collected from a farm that used contaminated water in Semarang, Magelang, and Salatiga (see Figure 1). The mercury concentration in each area was 5.54 ppm, 4.84 ppm, and 4.56 ppm respectively (Susanti et al., 2020). The concentration has already exceeded 0.001 ppm, the Indonesia International Standard of mercury threshold in water, and it was positively correlated with the mercury concentration in the water ways (SNI, 2009).

Figure 1. Locations of possible mercury contaminated areas in Central Java, Indonesia. This image was generated using the Map Customizer Website (https://www.mapcustomizer.com/).

One of the biggest sources of mercury exposure to humans is food consumption, where MeHg is absorbed through the human gut (Azevedo et al., 2012). The gastrointestinal tract can absorb around 90% of mercury in its organic form and 10% in its inorganic form (Rice et al., 2014). The absorbed MeHg has a strong affinity with thiol (-SH) and selenol (-SeH) containing proteins in the body (Nogara et al., 2019). Low molecular mass peptide, such as glutathione (GSH), contain thiols, whereas small number of proteins contain selenols. 25 genes in the human genome have been identified as selenoproteins that catalyze reactions of the detoxification of oxidants (Farina & Aschner, 2019). This interaction with thiol or selenol stabilizes MeHg, providing easy distribution to different organs through the bloodstream (Azevedo et al., 2012).

MeHg interaction with the thiol group from a tripeptide GSH (γ-l-glutamyl-l-cysteinylglycine), for example, can form MeHg-GSH complex by the catalyzation of glutathione-S-transferases (see Figure 2). The MeHg-GSH complex will be exported out of the cell and can be transported across liver canalicular membranes into bile as a major route of excretion (Farina & Aschner, 2019). The complex containing MeHg can circulate in the bloodstream, affecting different organs, such as the central nervous system (CNS) and the kidney (Santos et al., 2018). The MeHg-GSH complex decreases the toxicity of MeHg, but the thiol group from other proteins can easily react with the MeHg from the MeHg-GSH complex, allowing transfer of MeHg that leads to the oxidation of the sulfhydryl-containing proteins and changes in their function (Farina & Aschner, 2019). A direct interaction between MeHg and free L-cysteine is also possible, forming a cysteine-MeHg complex that can be easily taken by cells via a neutral L-amino acids carrier transport system (Farina & Aschner, 2019).



Sufficient active form of Selenium

Deficient active form of Selenium induced by MeHg

Figure 2. (a) The production of MeHg-GSH complex is catalyzed by glutathione-S-transferases (event a) that can be exported out of cell (event b) or stay inside the cell and interact with thiol group from other proteins (event c) (adapted from Farina & Aschner, 2019). (b) The production of glutathione peroxidase 1 in a normal state requires a sufficient amount of active selenium (event a), but it is downregulated when MeHg interacts with the active selenium and lowers the selenium concentration. This condition leads to a deficiency in active selenium that can be used for post-translational insertion in selenocysteine biosynthesis, which is important for the active site of GPx1, leading to a downregulation of GPx1 expression (event b) (Adapted from Fujimura & Usuki, 2020). This image was created with the BioRender website (https://www.biorender.com/).

Neurotoxicity and brain damage due to MeHg exposure is linked to the interaction with thiol or selenol groups in proteins that changes their structure, function, and redox state, resulting in increasing oxidative stress (Farina & Aschner, 2019). For example, MeHg can affect the production of glutathione peroxidase (GPx) (see Figure 2). The GPx protein family is a group of antioxidant enzymes that catalyze hydrogen peroxide reduction in the cell (Brigelius-Flohé & Maiorino, 2013). By doing this, the production of reactive oxygen species (ROS) can be properly maintained (Fujimura & Usuki, 2020).

Mining is another anthropogenic source of mercury emission in Indonesia. Many Indonesians are still practicing illegal ASGM, and they show an elevated mercury concentration in their blood, hair, and urine when compared to those who live in non-mining areas (Barkdull et al., 2019). The high risk of mercury exposure in mining usually happens during the screening and spawning process (Zulaikhah et al., 2020). After the gold containing ores are taken from underground or from open pit deposits, the ores are mechanically grounded to a fine powder, which will then be processed with liquid mercury. Inside the slurry containing fine gold ore powder, water, and liquid mercury, an amalgamation process occurs, forming mercury-gold alloy called amalgam and leaving other non-gold materials behind (Bose-O'Reilly et al., 2017). After that, smelting is conducted to vaporize the mercury and to obtain the gold from the alloy. The entire process is usually done with minimal standard safety precautions, leading to the risk of exposure to liquid mercury or inhalation of the toxic mercury vapor (Barkdull et al., 2019; Bjørklund et al., 2017; Bose-O'Reilly et al., 2017).

The skin contact with liquid mercury has the lowest risk due to its low bioavailability. As long as there is no open wound in the skin, a very tiny amount of mercury may enter the human body through the skin. This amount can not cause any harmful effects, and it will be washed away from the body through the urinary system. However, a chronic exposure (continuous or intermittent long-term contact) to the liquid mercury will eventually cause build-up in the body, leading to mercury poisoning symptoms. According to WHO, chronic exposure to elemental mercury levels of over 20 $\mu g/m^3$ in the air for several years can cause mild, subclinical signs of central nervous system toxicity (World Health Organization (WHO), 2017). Other than from constant skin contact, the most dangerous exposure comes from mercury vapor inhalation due to smelting or storing and handling mercury casually at home.

Although it poses the least danger, elemental mercury (liquid and vapor) is lipid soluble, allowing it to easily cross the blood-brain barrier in the body and the lipid bilayers of the cell membrane (Park & Zheng, 2012). Mercury exposure is particularly dangerous for pregnant women, infants, and young children, but its harmful effects really depend on how much mercury one is exposed to and the length of exposure (WHO, 2017). Acrodynia, or “pinks disease”, is shown by children who get exposed to mercury for a long time (Shandley & Austin, 2011). A very high mercury vapor concentration may cause acute bronchitis or pneumonitis (Park & Zheng, 2012). Adults may show symptoms like tremors, fatigue, irritability, increased stimulation, and memory loss, which have been linked to the disturbance in the central nervous system due to mercury poisoning (Zulaikhah et al., 2020).

ALGAE AND ITS GROWTH

In terms of taxonomic standing, the term algae is used for a diverse group of organisms that do not share a common origin, but follow multiple and independent evolutionary lines, non-cohesive, and artificial assemblage of photosynthetic organisms (Barsanti & Gualtieri, 2014). This definition may put plants into the same algal division, but the similarities between algae and plants are much fewer than their differences (Barsanti & Gualtieri, 2014). Algae lack true roots, stems, leaves, and well-defined vascular tissues; these are features shared with avascular lower plants, such as mosses, liverworts, and hornworts (Barsanti &

Gualtieri, 2014). The diversity of algae is very obvious in their dissimilar forms, which include microscopic single cells, macroscopic individual and aggregated multicellular, matted or branched colonies, or more complex leafy/blade forms (Barsanti & Gualtieri, 2014). In general, based on their harbored pigments, algae can be divided into three groups: green (Chlorophyta), red (Rhodophyta), and brown (Phaeophyta) (Pangestuti & Kim 2011; Patel et al., 2022). However, based on their size, algae can be grouped as micro and macroalgae. This review employs the term algae to refer to both micro and macroalgae.

Microalgae exist in water columns and on the sediment (Serôdio et al., 1997). They have the ability to move towards light to increase photosynthetic efficiency, migrating vertically in the water column depending on the time of the day (Serôdio et al., 1997; Suárez et al., 2008). Some microalgae can adhere to one another and form biofilms with bacteria that are important in the settlement of many marine invertebrates, such as bivalves and corals (Faimali et al., 2004; Roberts et al., 2010). Microalgae have been found to grow at a wide range of temperatures, with optimal temperatures usually found near the top of the range for a particular species; however, growth rate drastically decreases once the upper threshold is passed (Teoh et al., 2013). For example, a strain of *Chlamydomonas augustae* living in tropical regions was found to have a temperature growth range between 13 to 28 °C, but an optimum temperature range of between 23 to 28 °C (Teoh et al., 2013).

Macroalgae grow on rocky coastlines and are often exposed to air during low tides (Finke et al., 2007). These macroalgae produce reefs that vary greatly in structure and composition because of both biotic and abiotic conditions (Fulton et al., 2019). Some macroalgae species can form tall canopies, such as *Macrocystis pyrifera*, that can grow to over 30 meters (Cavanaugh et al., 2011; Tompkins & Wolff 2017). Others remain relatively small, such as *Ulva spp.*, and grow at lower and mid-littoral zones of the rocky shores, such as *Corallina spp.* (Cavanaugh et al., 2011; Tompkins & Wolff, 2017). Macroalgae form expansive reef habitats for many organisms (Fulton et al., 2019).

Many factors contribute to healthy algae growth, such as nutrient availability, light intensity, pH, temperature, and wave flow. In nature, the highest algae growth is found in areas where upwelling occurs (Tompkins & Wolff, 2017; Paula et al., 2020). Upwelling is the flow of water away from the shoreline that results in the upward movement of deeper, colder, and nutrient-rich water to the surface, driving algae growth (Paula et al., 2020; Tompkins & Wolff, 2017). Rivers can also contain fertilizer and untreated sewage that contribute to high and uncontrolled algae growth (Suwarno et al., 2013).

The effect of light on algae growth is due to the different pigments that absorb specific wavelengths of light. The red and brown algae are capable of living deeper in the water column because they contain accessory pigments that are capable of capturing light that penetrate deeper into the water (Osório et al., 2020; Pangestuti & Kim, 2011). The difference in light penetration into water results in an evident distribution of algal species, with the green algae generally occupying the upper intertidal area, whereas the red and brown algae being found in deeper water columns (Leukart & Lüning, 1994). The irradiance and photoperiod are also important factors that contribute to an optimum algae growth efficiency (Singh & Singh, 2015).

The optimum pH for most algae species is ~8.2 to 8.7 pH in an aquatic environment. This is largely affected by the equilibrium between the dissolved atmospheric carbon dioxide (CO_2) and calcium carbonate ($CaCO_3$) found in aquatic structures. When H_2O reacts with CO_2, carbonic acid (H_2CO_3) is produced, which will then dissociate into bicarbonate (HCO_3^-) and hydrogen ion (H^+) (Hofmann & Bischof, 2014) (see Figure 3). $CaCO_3$ naturally dissociates into calcium (Ca^{2+}) and carbonate ions (CO_3^{2-}). CO_3^{2-} react with H^+, from the dissociation of H_2CO_3, to become HCO_3^-. Under normal conditions, HCO_3^- is able to return into its constituent compounds. However, the increasing concentration of atmospheric carbon from human

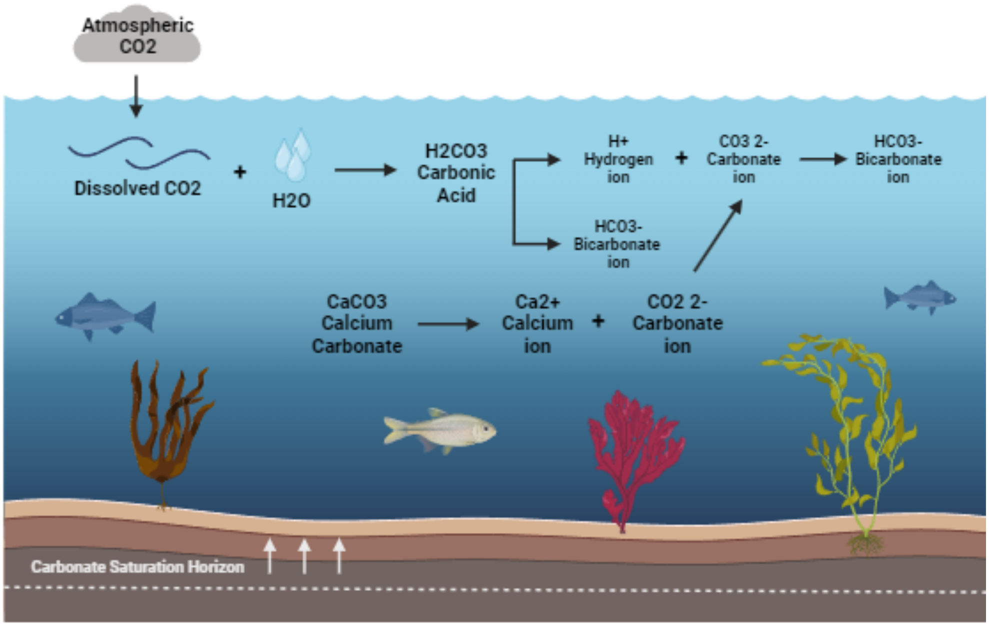


Figure 3. The ocean acidification mechanism adapted from Figuerola et al. (2021). This image was created with the BioRender website (https://www.biorender.com/).

activities will continue to drive the reaction forward, taking away CO_3^{2-} which is essential for the calcification of many marine organisms including many algae (Hofmann & Bischof, 2014). On the other hand, other algae species that do not require the carbonate ions may benefit from lower pH, which could eventually result in a population shift that favors non-calcifying algae (Ho & Carpenter, 2017).

Temperature affects algal productivity in photosynthesis, protein and lipid synthesis, and reproduction (Singh & Singh, 2015). The effects of temperature are mostly species dependent (Singh & Singh, 2015). For example, microalgae, although can be found in many different environments, some species have optimum temperatures between 44-51°C, whereas others can have a wide temperature range from 5-30°C (Singh & Singh, 2015). A Galapagos based study revealed that not only do algae exist at shallow waters, but they also live at deeper depths, where a cooler and more stable temperature is available and preferred by the algae (Tompkins & Wolff, 2017). An important factor affecting the ocean temperature is the El Niño/La Niña Southern Oscillation. These climate patterns have implications for algae growth because they change the average sea surface temperature and affect upwelling conditions (Hernández-Carmona et al., 2011; Tompkins & Wolff, 2017).

Waves and currents influence different variables that affect algae growth, including morphology, carbon absorption, larvae/spore dispersal, mass transfer of nutrients from water to algae, and photosynthesis (Ho & Carpenter, 2017). Fast currents are expected to increase the rate of nutrient uptake because more nutrition is being supplied per unit of time, thus increasing the growth of algae (Ho & Carpenter, 2017; Wang et al., 2016). Algae adapt to this condition by producing leaf blades that are more streamlined to prevent shearing stress, whereas algae that live in slower-moving water adapt by increasing their surface area (smaller leaf blades) to increase nutrient uptake from the water (Ho & Carpenter, 2017).

As an archipelago, Indonesia has all the physical characteristics capable of culturing algae for the purpose of absorbing heavy metals, particularly mercury. Warm water in the Indonesian ocean comes from the north and south Pacific Ocean currents (Chen et al., 2004). The currents become concentrated in the Western Pacific Warm Pool due to the geography of the Indonesian archipelago (Purba and Khan, 2019;

Chen et al., 2004). Upwelling has been found to occur every year due to the movement of winds during the Asian Australian Monsoon seasons, allowing for nutrient-rich waters to rise to the surface (Purba and Khan, 2019) (Purba & Khan, 2019; Wirasatriya et al., 2021). Furthermore, Indonesia has approximately 12 hours of sunlight per day, which allows the cultivation of algae all year round. However, it is important to first select potential algae candidates that will best suit the absorption of heavy metal so that their optimum growth requirements can be narrowed down (Csavina et al., 2011).

It is also possible to use algae grown in freshwater. However, these algae are affected by a number of different environmental factors, such as salinity and shading (from terrestrial vegetation or turbidity). Growth rates of freshwater algae have been found to reduce with increasing salinity, and increase in shade reduces photosynthetic efficiency of algae (Herbst & Castenholz, 1994; Sturt et al., 2011). Species-specific studies need to be conducted to determine the ideal growth requirements for each culture species.

ALGAE AS A BIOSORBENT

Algae are extremely adaptable organisms that can grow in adverse environments such as extreme temperature and pH, as well as low nutrition levels, giving them an advantage over other potential microorganisms for biosorbents like bacteria and fungi (Adewuyi, 2020). Algae can utilize cellulose, pectins, lignin, but most importantly fucoidan and alginate: compounds found in their cell walls to remove mercury (Anastopoulos & Kyzas, 2015; Zeraatkar et al., 2016). The cell wall in algae also contains negatively charged functional groups, such as SH^-, COO^-, RNH_2^-, RS^-, RO^-, and OH^-, that can act as binding sites for heavy metals such as mercury (Al-Homaidan et al., 2018; Ankit et al., 2022). These chemical groups are abundant in the algae cell surface and the cytoplasm, particularly in vacuoles (Ankit et al., 2022; Wahid & Khan, 2020). The mercury ions in the water are exchanged with other ions such as Na^+, Ca^{2+}, and K^+ found on the surface of the algae cell wall, leading to the removal of this heavy metal by covalent or ionic bonding (Ankit et al., 2022). The entire process is highly dependent on the physicochemical property of the cell wall and the metal selectivity (Ankit et al., 2022). Once taken, the mercury will then be metabolized by the algae.

In terms of mercury metabolism, microalgae are rich in peptides such as the cysteine-rich peptide (glutathione), some lipopolysaccharides, phytochelatins, and metallothioneins, enabling them to bond with mercury to produce an organometallic complex, which is usually delivered to vacuoles to control the heavy metal concentration in the cytoplasm (Kaur et al., 2019). The organometallic complex production is a metabolic process that can neutralize any potential harmful effects of heavy metals in algae (Kaur et al., 2019). Phytochelatins are oligomers of glutathione that act as chelators, which are important for heavy metal detoxification, and metallothioneins are synthesized as a response to the presence of mercury. They both enable algae to survive high mercury concentrations (Souza et al., 2012). Other than that, these peptides have high adsorption kinetics that can metabolize mercury rapidly (Kumar et al., 2020).

The adsorption capacity of algae determines their function as mercury biosorbents (Nazal, 2018). To obtain a better adsorption capacity, physicochemical modifications could be applied to the algae biomass (Adewuyi, 2020). Several reagents, such as alkalis, organic compounds, acids, enzymes, salts, and EDTA, can be used to pretreat the algae biomass. According to Bilal et al. (2018), the most suitable reagent for algae to increase their mercury adsorption is by treating them with acid. Acid can dissolve the polysaccharide compounds of the cell wall to a certain extent, resulting in the formation of additional binding sites (Kaur et al., 2019).

The reagents treatment must also consider the alga species for optimum results. For example, when brown algae *Fucus vesiculosus, Cystoseira myrica,* and *Scenedesmus obliquus* were pre-treated with HCl, the only species that showed a significant adsorption increase was *C. myricia*. There was no effect observed in *F. vesiculosus*, whereas *S. obliquus* was affected negatively (Khoroshutin et al., 2021). Therefore, for an industrial algae biosorbent, studies that can be used to find the most suitable reagents for a specific alga species must also be considered to get optimum biosorbent activity.

Different cell wall compositions affect an algae's biosorption capacity, with some algae being better suited to remove certain metals from water (Bilal et al., 2018; Romera et al., 2007). Many findings have suggested that brown algae had the greatest biosorption ability (Ordóñez et al., 2023; He & Chen, 2014). Their cell wall contains several components that help them achieve this. First is the presence of alginate, which has a carboxyl group that can be deprotonated to form negatively charged carboxylates. These carboxylates can then interact with the positively charged metal ions (Gao et al., 2020; He & Chen, 2014). The second component is the amino and sulfhydryl groups that are present, providing additional binding sites for metal ions and increasing affinity for heavy metals such as mercury, cadmium, and lead (Ordóñez et al., 2023). On the other hand, the cell wall of red algae is composed of polysaccharides such as agar and carrageenans. The negatively charged sulfate groups in agar and carrageenan provide binding sites for positively charged metal ions through electrostatic interactions (Khotimchenko et al., 2010). The cell wall of green algae is mostly composed of cellulose, pectin, and glycoproteins, but lack fucoid compounds resulting in a weaker binding of heavy metals.

However, several studies have found that brown algae may not be the most efficient (Cordova & Muhtadi, 2017). One study found the green algae, *Ulva lactuca*, to be more effective in mercury removal compared to the brown algae, *F. vesiculosus*, because of their difference in surface area and metabolism (Henriques et al., 2015). Furthermore, *U. lactuca* had a higher growth rate compared to *F. vesiculosus* and was easier to maintain in the laboratory, resulting in greater removal overall (Henriques et al., 2015). Therefore, choosing the right algae is important as some physical and molecular properties of algae may work more efficiently than others depending on the working environment (Romera et al., 2007).

Green algae (Chlorophyta) as biosorbent

Green algae contain chlorophyll *a* and *b* that absorb wavelengths of blue and red lights, reflecting an observable green light as a result (Osório et al., 2020; Pangestuti & Kim 2011). They also contain carotenoids, which are accessory pigments that are useful in lowering the reactive oxygen species that disrupts cellular metabolic activities (Delwiche & Palmer, 1997; Pangestuti & Kim, 2011). Green algae have an ability to enhance the accumulation of Hg^{2+} due to the physiological configuration of its chloroplast, as revealed by an *in vitro* study by Quiroga-Flores et al. (2021). Green algae grow optimally in temperature between 24-28°C and at a pH of 7.5 (Munir et al., 2015). They grow better under artificial fluorescent light compared to direct and indirect sunlight.

Ji et al. (2011) showed that fresh *C. fracta* has an adsorption capacity of 0.228 mg Hg^{2+} per gram of living algae was grown at 18°C and a pH of 5.0. Other than that, this species had a 98% efficiency of removal when the concentration level of Hg^{2+} in aqueous solutions was low. This type of algae may be an environmentally friendly and cost-effective option for water body treatment in residential areas due their great adaptability, low cost, ease of growth and harvest, and wide distribution (Topal et al., 2020).

Ulva intestinalis is fairly abundant in Pantai Tanjung Layar, which is located in Kabupaten Lebak, Banten, Indonesia (see Figure 4). Fabre et al. (2021) showed that *U. intestinalis* can be an excellent biosorbent, as it has an adsorption capacity of 1.888 mg Hg^{2+} per gram algal biomass at 22°C and pH of 8.5. This alga had a removal efficiency of 97.3% with high water content, indicating a possible use for industrial water body treatment where the liquid waste is saturated.

Oedogonium sp. is abundant in Sungai Asik, which is located in Kabupaten Pasaman, North Sumatra, Indonesia (see Figure 4). Lanza et al. (2017) showed that *Oedogonium* sp. was the best species in accumulating methylmercury from Lake Uru-Uru in Bolivia. Quiroga-Flores et al. (2021) revealed that *Oedogonium* sp. had an adsorption capacity of 0.003 mg MeHg per gram algal biomass at pH 6.8 and a temperature of 16.5°C.

Chlorella vulgaris is another green microalga species with a great mercury biosorbent potential. In Indonesia, this alga species is cultured in Pakan Alami Budidaya Air Payau Laboratory, Balai Besar Pengembangan Budidaya Air Payau in Jepara, Central Java, Indonesia (see Figure 4). Kumar et al. (2020) used algae from the algae culture collection of the Indian Agriculture Research Institute in New Delhi to remove the mercury in aqueous solution at pH 6 and 35°C. They found out that *C. vulgaris* had an adsorption capacity of 52.09 mg per gram of algal biomass.

A green microalga, *Chlamydomonas reinhardtii*, is another potential mercury biosorbent species. This alga has been used for treatment of heavy metal contamination in an aqueous solution where it can tolerate a broad range of heavy metal, including gold, lead, mercury, nickel, cadmium, cobalt, copper, silver, and zinc (Hanikenne, 2003). Furthermore, *C. reinhardtii* can be genetically modified (transgenic algae) to improve its ability to isolate hazardous heavy metals in water pollution (Cai et al., 1995). From a sample of *C. reinhardtii* isolated from Kızılırmak River in Türkiye, it was shown that this species had a maximum biosorption capacity of 72.2 ± 0.67 mg per gram algal biomass (Tüzün et al., 2005).

Figure 4. Location of green algae used as mercury biosorbent throughout Indonesia. This map was generated by the Map Customizer website (https://www.mapcustomizer.com/)

Red Algae (Rhodophyta) as biosorbent

In addition to containing chlorophyll pigments, red algae also contain phycobilins, an accessory pigment that absorbs green wavelengths, resulting in a red appearance (Osório et al., 2020; Pangestuti & Kim, 2011). Phycobilins are useful for absorbing light at deeper depths because of the decrease in available wavelengths. However, red algae may actually be able to adapt to different light intensities, resulting in lower phycobilin yield (Leukart & Lüning, 1994). Red algae cell walls are composed of carrageenan, sulfated polysaccharides, agar, celluloses, and glycoproteins with sulfate, amino acids, hydroxyl, and carboxyl groups (Znad et al., 2022). Heavy metals will bind to the alga more effectively if there are more functional groups in the cell wall (Bilal et al., 2018). Due to its cell wall composition, red algae have biosorbent potential.

Generally, red algae grow optimally at 20^{o}C and at a pH of 6 - 8.5 (Perona et al., 2011). According to Cordova & Muhtadi (2017), the red algae *Gracilaria salicornia* may absorb up to 41.45 µg Hg^{2+} per kilogram biomass. This alga has also been isolated from the North, East, South, and West regions of Pari Island in DKI Jakarta, Indonesia (see Figure 5). Also, part of the red alga species, *Gracilaria gracilis* was shown by Henriques et al. (2015) to have an adsorption capacity of 0.047±0.003 g per gram biomass and 87% removal effectiveness at a pH of 5. Despite the fact that the study was not conducted in Indonesia, *G. gracilis* can be easily found on the south coast of Java (Meinita, 2021). The other biosorbent candidate for red alga is *Gracilaria cortica*, which was shown by Esmaeili et al. (2015), in their study of *G. cortica* isolated from the Persian Gulf on Queshm Island, to have a 90% removal effectiveness at a pH of 5 and temperature of 21^{o}C - 25^{o}C.

In addition, Zaib et al. (2016) have also shown that a red microalga, *Porphyridium curentum*, has a reliable biosorbent activity for Hg^{2+}. *P. curentum* showed a removal capacity of 2.62 mg per gram biomass at a pH of 7 (Zaib et al., 2016). This alga species has also been cultured in the Oceanographic Research and Development Center of the Indonesian Institute of Sciences (Prasetyo et al., 2015).

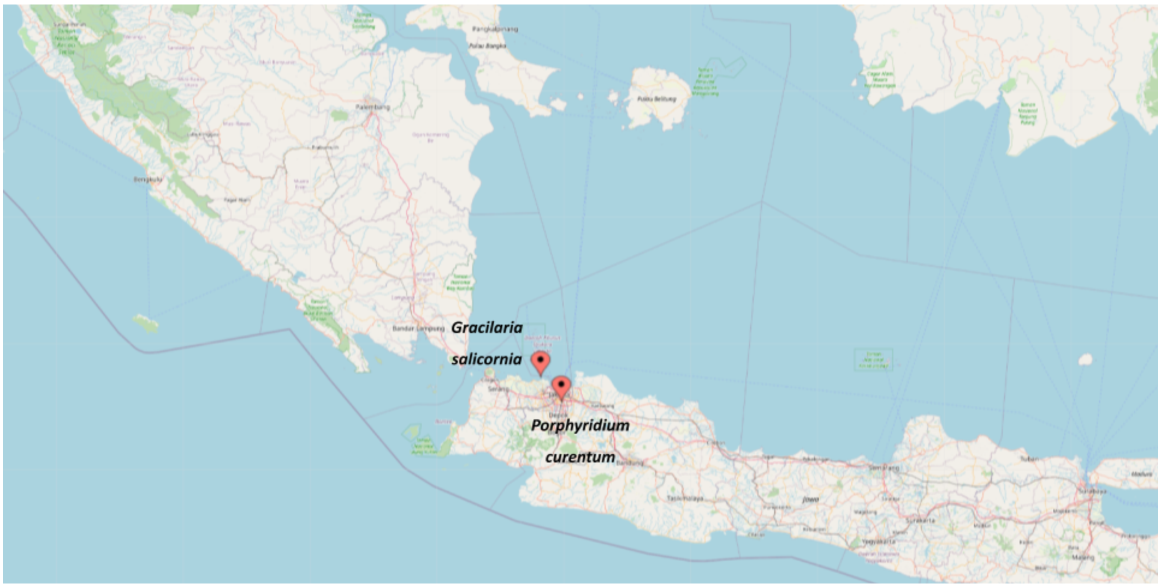


Figure 5. Location of red algae used as mercury biosorbent throughout Indonesia. This map was generated by the Map Customizer Website (https://www.mapcustomizer.com/)

Brown algae (Phaeophyta) as biosorbent

Brown algae were derived from a red algae precursor and contain similar pigments (Delwiche & Palmer, 1997). However, the brown coloration is due to the presence of carotenoid fucoxanthin, a compound whose structure and composition can differ among different brown algae species (Pangestuti & Kim, 2011). *Sargassum glaucescens* is an example of brown alga with a great potential capacity of biosorbents (Esmaeili et al., 2015; Mokone, 2018; Saldarriaga-Hernandez et al., 2020). Esmaeli et al. (2012) showed that *S. glaucescens* reached its optimum adsorption of Hg^{2+} at different pH and time points. For example, at 90 minutes, the optimum pH for the adsorption of Hg^{2+} was 5, while at 30 minutes, the optimum pH was 7. Esmaeli et al. (2017) showed that *S. glaucescens* had Hg^{2+} adsorption efficiency at 95.1% when situated in optimum conditions with the adsorption rate of 147.5 mg per gram biomass.

Sumandiarsa et al. (2020) stated that *Sargassum sp.* is abundant in several parts of Indonesia, particularly in western Indonesia. A study by Putri & Syafiqa (2019) showed that *S. crassifolium* was found in the water of Kotok Besar Island (see Figure 6) and could be potentially used as a biosorbent for heavy metals in industrial water bodies. At a pH of 2-3 or 9 and a contact duration of 60 minutes, this species selectively absorbed 37.78% - 99.05% of heavy metals from industrial water bodies (Putri & Syafiqa, 2019). For Hg, the greatest adsorption capacity was at around 75% - 99.05% (Putri & Syafiqa, 2019).

Cystoseira compressa is another brown alga which has a potential bioaccumulation activity (Pennesi et al., 2012). According to Oucif et al. (2020), this alga species has the potential of taking up mercury up to 6.48e-8 mg per gram biomass, with an optimum temperature of up to 35°C and pH of 5.0 to 9.1. *Hormophysa triquetra* is another species of brown algae species for biosorbent with the ability to take up an amount of 2.15e-5 ± 9.59e-6 mg mercury per gram biomass (Cordova & Muhtadi, 2017). Meiyasa et al. (2020) showed that *H. triquetra* was used in the Moudolung waters in East Sumba (see Figure 6). This alga species can work well at 29°C, a pH between 8.3 - 8.4, and a dissolved oxygen (DO) concentration of 7.7 - 8.1 mg/L (Meiyasa et al., 2020).

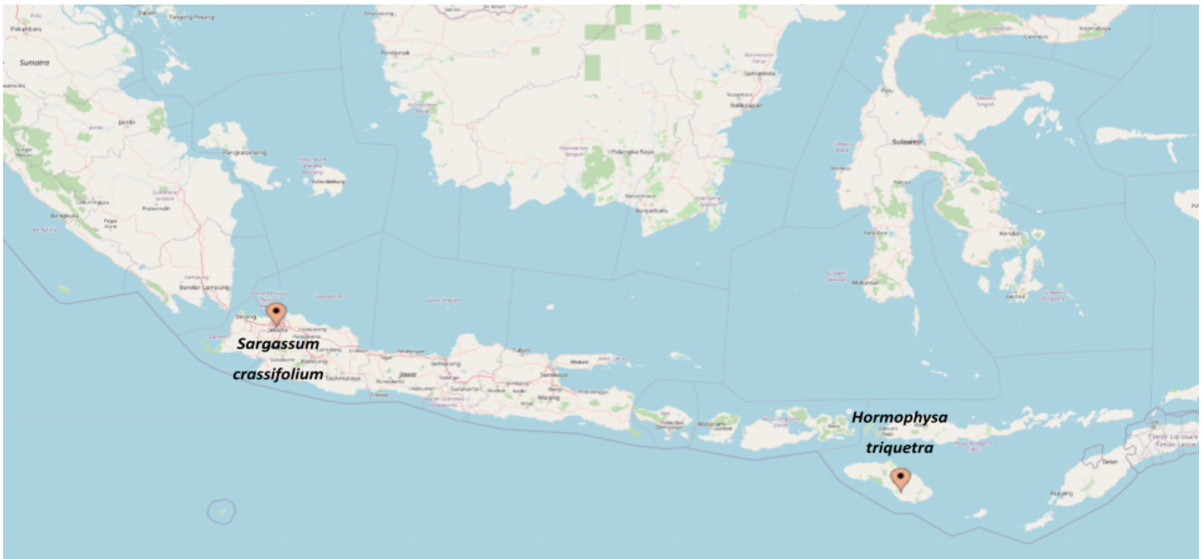


Figure 6. Location of brown algae used as mercury biosorbent throughout Indonesia. This map was generated by the Map Customizer website (https://www.mapcustomizer.com/)

THE GENETIC MODIFICATION OF ALGAE FOR BIOSORPTION

The resilience of algae to high heavy metal concentrations has stimulated research into the molecular mechanisms underpinning algal heavy metal tolerance and removal capacity (Puente-Sánchez et al., 2018). Further research has shown that algae can isolate metal ions via the intracellular polyphosphates and external polysaccharides that chelate metal ions. In addition, when algae were exposed to stressful situations like water scarcity, severe temperatures, salinization, nutritional insufficiency, it was observed that specific metal-binding proteins were created as a counter-response (Nowicka, 2022). This emphasizes the prospect of enhancing heavy metal removal from the environment by overexpressing or modifying algal heavy metal resistance systems (Cheng et al., 2019).

The foremost molecular technique, known as cell-surface display technology, is to overexpress trace metal binding components at the algal surface, such as metal-binding proteins and compounds of phytochelatins, proline, metallothionein, and glutathione (Bae et al., 2022; Cobbett & Goldsbrough, 2002). Using a cell surface display approach, He et al. (2011) revealed a membrane-anchored metallothionein polymer in *C. reinhardtii: A significant* improvement in the mercury removal capacity of the transgenic microalga against its wild type.

Because of their ease of manipulation and rapid development, yeasts and bacteria provide a source of data on cell-surface engineering implementation that may be used to guide comparable investigations in microalgae (Kuroda et al., 2001). Algae have been demonstrated to have beneficial qualities for heavy metal recovery, such as the inducible production of heavy metal binding phytochelatins and their genetic manipulation capability (Rajamani et al., 2007). Therefore, genetic modification of algae can be achieved using two techniques: gene overexpression and the creation of transgenic algae (Cheng et al., 2019).

In overexpressing genes, the discovery of genes encoding metalloregulatory proteins is critical for the genetic engineering of algae (Danouche et al., 2021). For example, a green microalgae species is found to have several metal-tolerance protein (MTPs) genes in one type of species. Each MTPs gene contributes to a different metal-binding ability. However, overexpressing only one of the MTPs genes may not be enough to boost algal metal tolerance (Ibuot et al., 2017).

The *Bacillus megaterium* strain MB1 merA gene, which codes for mercuric reductase (merA) and mediates the conversion of Hg^{2+} to its volatile elemental Hg^0, was introduced into the eukaryotic microalga *Chlorella sp. DT.* Hygromycin B and $HgCl_2$ were used to select the transformed *Chlorella sp*. cells. Polymerase chain reaction amplification demonstrated the presence of the merA gene in the genomic DNA of transgenic strains, and Southern blot analysis verified the gene's stable integration (Huang et al., 2006).

The merA transgenic strains had double the capacity of eliminating Hg^{2+} compared to the wild type (Dai et al., 2009). In the presence of a hazardous quantity of Hg^{2+}, the merA transgenic strains outperformed the wild type in terms of growth rate and photosynthetic activity. Superoxide dismutase expression levels in transgenic strains were lower when cultured in Hg^{2+} than in the wild type, indicating that the transgenic strains experienced less oxidative stress. All of the findings suggest that the merA gene was successfully inserted into the transgenic strains' genomes and was functionally expressed to encourage the excretion of Hg^{2+} (Huang et al., 2006).

Moreover, in strains of microalgae *Selenastrum minutum, Chlorella fusca, and Galdieria sulphuraria*, merA gene was also expressed, allowing the biotransformation of Hg^{2+} to elemental Hg and metacinnabar (β-HgS) (Ranjbar & Malcata, 2022). This method's applicability was previously shown to increase the tolerance of different plants to mercury. Additionally, it was found that simultaneous expression of the merA and merB genes, codon optimization, and endoplasmic reticulum (ER) targeting of MerB (organomercurial lyase) increased Hg^{2+} and R-Hg^+ biotransformation in transgenic plants by up to ten fold (Lyyra et al., 2007). Organomercurial lyase mediates the protonolysis of organic mercury to Hg^{2+}, while mercuric reductase reduces Hg^{2+} to Hg^0. As a result, overexpression of cytosolic MerA and ER-located MerB using (microalgae-specific) codon optimized genes may prove to be a successful strategy to increase the HM-biotransformation capacity of microalgae (Ruiz & Daniel, 2009).

The merP in the periplasm is responsible for directing metal ions, preferably ionic mercury, to those inner membrane metal transporters. Keep in mind that the mer operon also contains the coding genes for three inner membrane transporters (merC, merT, and merF) involved in the transport of mercury into the cytosol (Silver & Phung, 2005). Therefore, the ability of microalgae to remove mercury is supposedly improved by the expression of the merP gene or the fusing of merC/T/F to a metal chelator (as membrane-bound Hg^{2+} trap) (Ruiz & Daniel, 2009). It may be safer to sequester ionic mercury inside the cell by attaching to a chelating molecule, given the environmental concerns regarding the excessive release of Hg^0 as volatile metabolites into the atmosphere by such designed organisms.

LIMITATIONS OF USING ALGAE AS BIOSORBENT

Despite the countless benefits that algae have in their application as biosorbent to remove several heavy metals, there are still some limitations to their use. The drawbacks include their small size, inefficient mechanical strength, and the complication of effluent and biomass separation (Gadd, 2009). Although Salam (2019) stated that these complications have been reduced with the use of cross-linked biomass, a new issue arises from this novel usage: the slowing down of metal removal rate as well as the drop down in the sorption capacity.

In Indonesia, some algae such as *G. gracilis* and *C. compressa* have not been used as a biosorbent, necessitating further research to know its capability in absorbing mercury. There are still too many species of algae that have not been experimented with for their mercury absorption capacity. Moreover, when a species is introduced to a new location, there is always a possibility that the introduction fails to meet the expected optimal adsorption rate.

Further studies must also be considered to understand the long-term risks of algae biosorbents. The average growth rate of algae is 3.1×10^5 cells per mL per day, which can be considered to be rapid. If algae can still grow exponentially in the presence of heavy metals like mercury, there is always a risk of algae eventually becoming a harmful invasive species toward the native species. A possibility of mercury bioaccumulation in the food chain may also occur as a long-term risk of algae biosorbents.

CONCLUSION AND FUTURE RECOMMENDATIONS

Over the years, many countries with high heavy metals contamination in their water have been keeping up with algae biosorbent, including Indonesia. Algae provide potential usage as a biosorbent due to their high efficiency, ability to absorb heavy metals at a large capacity, and low-cost of production. Aside from the successful trials of the listed algae species in treating mercury contamination in the Indonesian water bodies, a species like *G. gracilis* was not tested in the study. There are many other algal species which have not been tested. This opens up the possibility of exploring the wide range of algae species that can be brought into a large-scale treatment of industrial water wastes. Future research could focus more on developing a more efficient alga species by improving their genetic trait through genetic modification. In addition, further investigations should consider the viability of utilizing algae as a long-term water treatment over other combinations of chemical and physical treatments. Questions such as “Is it more effective?” or “Can it be a better solution?” would be the base mark in the development of water treatment using algae in the future.

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