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Lead Reduction on Polluted Water and Sediment through The Use of *Anadara granosa* shells and *Monostroma nitidum* Biological Filters

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Abstract

Industrial wastewater treatment holds a very vital role in filtering toxic materials and improving the quality of industrial wastewater before being released into public waterways, so that the water ecosystem balance can be maintained. This study aims to identify Pb concentration on polluted water and sediment from an offshore oil drilling area near Camplong Beach, Madura, and also make efforts to find out the potential *Anadara granosa* shells and *Monostroma nitidum* seaweed biofilters that exist in that place so as to suppress the adverse effects of toxic materials that arise. The method used was an experimental research design that aims to systematically obtain information to describe a lead concentration in polluted water and sediment, and also evaluate the presence of *A. granosa* shells and *M. nitidum* as a potential biological filter. The results of the analysis of lead in *A. granosa* shells, *M. nitidum* biofilter, water, and sediment indicated that there was a significant correlation, where the shells of *A. granosa* and *M. nitidum* can absorb lead until 36.59% and 8.79%, respectively, from which the initial concentrations of lead in sediment and water were 5.57±1.58 and 0.004±0.001 mg/kg respectively. The existence of algicidal substances of HDTA, ALA, and ODTA from *M. nitidum* can suppress the growth of the dinoflagellate *Chattonella marina*. The quantification analysis of fatty acid composition showed that *M. nitidum* is dominated by PUFA as much as 66% of total fatty acid, and has algicidal substances of HDTA, ALA, and ODTA of 61.53 mg/100 g.

INTRODUCTION

Industries using lead as raw or supporting materials could potentially become the main source of lead pollution, such as the foundry and refining industry, battery companies, fuel companies, cable companies, and chemical coloring companies. Foundry and refining companies produce both primary and secondary lead derived from metal scraps. Battery companies also use lead, especially lead-antimony alloys and lead oxides, as raw materials. Fuel companies produce lead (Pb) in the forms of tetra ethyl lead and tetra methyl lead which are used extensively as an anti-knock agent in fuel; thus, both the fuel companies and the fuel produced become the source of Pb pollution. Cable

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companies require Pb for cable coating, even though the use of Pb is getting less and less. Nonetheless, they still use metal mixtures of Cd, Fe, Cr, Au, and arsenic, which also pose threats to living beings. In the chemical coloring industry, Pb is often used because its toxicity is relatively lower than other metal-based pigments. The red color of paint, for example, usually comes from the red lead while yellow color uses lead chromate.

Wahyudi *et al*. (2014) showed that the results of the analysis of Pb levels in sediments at South Beach of Bangkalan Regency have exceeded the quality standard of marine waters (0.230 ppm > 0.005 ppm). Pb levels in each beach are Labang Beach 0.187 ppm, Kwanyar Beach 0.349 ppm, and Modung Beach 0.154 ppm. Furthermore, the results of Pb levels analysis in water on the South Beach of Bangkalan Regency has also exceeded marine water quality standards (0.129 ppm > 0.005 ppm). Pb levels in each beach are Labang Beach 0.085 ppm, Kwanyar Beach 0.154 ppm, and Modung Beach 0.150 ppm.

The increasing level of heavy metals in waters can be caused by other industrial waste, mine waste, farming waste, and domestic waste, which contain heavy metals (Verma and Dwivedi, 2013). Heavy metal levels above normal in waters (10^{-5}- 10^{-2} ppm) will produce toxins; a continuous increase of heavy metal levels will be followed by an increase of heavy metal contents of biota (especially filter feeder and demersal organisms) that eventually ends up as pollution (Nunes *et al*., 2014).

Industries that produce Pb as waste have used wastewater treatment systems that can filter and reduce poisonous substances and improve the quality of wastewater before being released to the public waters as an effort to maintain the water ecosystem balance. However, in reality, these wastewater treatment systems are often found to be ineffective as the waste still contains high levels of heavy metals which have lowered the water ecosystem quality (Barakat, 2011). Heavy metals in waters have an impact on aquatic organisms as well as humans. One of the impacts was the mass fish mortality that occurred in Jakarta Bay in 2004. The heavy metal content in Jakarta Bay is high, making it dangerous for aquatic organisms (Rochyatun and Rozak, 2017). Heavy metals that enter the human body are also dangerous for human health. Heavy metals can block the work of enzymes so that the body's metabolism is disrupted, causing cancer and mutations. Some heavy metals are very dangerous for humans, among others lead, copper, mercury, cadmium, and chrome (Effendy *et al*., 2018).

Generally, the main components of an industrial wastewater treatment system are biological, chemical, and physical filters (Raut and Gotmare, 2019). The main focus of the research was on shell engineering and seaweed as biofilters, considering their easy applications and ability to biologically absorb lead toxicity or biosorption.

The shells of *Anadara granosa* contain $CaCO_3$ which can bind heavy metals in water. The *Sargassum filipendulaa* seaweeds are also able to perform lead biosorption (Vieira *et al*., 2007). Interestingly, research conducted by Dhokpande and Kaware (2013) and Perryman *et al*. (2017) suggest that microorganisms such as bacteria, algae, fungi, yeast, and seaweed can also be used as biofilters, function as biosorption of heavy metals, and have algicidal substances that can suppress the growth of Dinoflagellates (Inaba *et al*., 2014). The bioactive agents of Chlorophyta *Ulva fasciata* that have been explored, among others, are unsaturated fatty acids, such as hexadeca-4,7,10,13-tetraenoic acid, linoleic acid (HDTA), α-linolenic acid (ALA), and octadeca-6,9,12,15-tetraenoic acid (ODTA) which are very potent for the growth and survival of Dinoflagellates (Alamsjah *et al*., 2007). Chlorophyceae types of seaweed are also known to be able to absorb several polluting materials (*biosorption*) in a water environment (Jeong *et al*., 2000).

This study aims to identify Pb concentration on polluted water and sediment

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from an offshore oil drilling area near Camplong Beach, Madura, and also make efforts to find out the potential *A. granosa* shells and *M. nitidum* seaweed biofilters that exist in that place to suppress the adverse effects of toxic materials that arise.

METHODOLOGY

Ethical Approval

Not applicable.

Place and Time

Samples of *A. granosa* shells, *M. nitidum* seaweed, water, plankton, and sediment were taken from the area of the offshore oil drilling rig in Camplong Beach, Madura. Sample collection was carried out in January – March 2023.

Research Materials

The main materials in this research were wastewater and sediment from an offshore oil drilling area near Camplong Beach, Madura, dinoflagellate *C. marina* which is found to grow near the wastewater zone of the oil drilling area, *A. granosa* seashells, and *M. nitidum* seaweed. The materials used to detect *M. nitidum* fatty acid composition were methanol, HCl, CH_2Cl_2, standardized methyl ester combined-fatty acids of C_8-C_{22} and C_{14}-C_{22} (Supelco), and Na_2SO_4. The materials used to analyze the level of heavy metals absorbed by *A. granosa* and *M. nitidum* were distilled water, HNO_3, $HClO_4$, $SnCl_2$, $HgCl_2$, standard solvents (Pb, Cd, Cu, Cr, Mn, Ni, Fe, Zn, Hg), H_2SO_4, seawater, methanol, Autoclaved Sea Water (ASW), and Enriched Seawater Medium (ESM).

The instruments used in this research were FL40SD fluorescent lamps (Toshiba), Erlenmeyer flasks, microplates, plastic bags, filter paper no. 2 (Advantec), binocular and trinocular microscopes (Olympus), a hemocytometer, a concentrator (Taitec), a centrifuge (Eppendorf), column chromatography, micro pipettes (Gilson), a J-6B centrifuge (Beckman), an experiment tub, a blower, pH pens, a refractometer, flask bottom aerators, an aerating hose, tips, petri dishes, GC-2014, and Atomic Absorbance Spectrophotometric (AAS).

Research Design

Research design using descriptive method is to give insight into the amount of Pb concentration level of heavy metals in the marine ecosystem especially those absorbed in the body of *A. granosa* shells, thallus of *M. nitidum* seaweed, water, plankton, and sediment. Finally, the review examines lead reduction in polluted water and sediment through the use of *A. granosa* shells and *M. nitidum* biological filters.

Work Procedure

Sample Collection

Samples of 1000 L water and 40 kg sediment were taken from the area of the offshore oil drilling rig in Camplong Beach, Madura. The sediment was taken with a stainless-steel grab. *A. granosa* shells and *M. nitidum* seaweed were collected during low tide. The morphological characteristics and species anatomy were examined with an optical microscope to isolate and identify the specimens needed. The collections of *A. granosa* shells and *M. nitidum* seaweed from intertidal areas were conducted during the first month of the research. Ecological damages during sample collections were minimized. All samples were taken to the laboratory in plastic bags that contained seawater to avoid evaporation. Then, they were washed with ASW to separate potential contaminants. *Chattonella marina* Dinoflagellate were identified to grow in the contaminated water taken from the offshore oil drilling area.

Pre-treatment of *Anadara granosa* Shells and *Monostroma nitidum* in an Indoor Condition

The method to perform this research was followed by Benkendorff (1999) with minor modification. The shell of *A. granosa* and *M. nitidum* seaweed originating from Pb-polluted waters from the area of the offshore oil drilling rig in Camplong

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Beach, Madura were further observed in the laboratory. Initial culture of *M. nitidum* was conducted for 30 days and after that they were moved to a treatment aquarium containing water and sediment obtained from the offshore oil drilling area. The total volume of A. granosa shells used in every treatment medium was 100 g/L, while the volume of *M. nitidum* was 400 g/L.

Biosorption Treatment of *Anadara granosa* Shells and *Monostroma nitidum* Against Lead Toxicity

The duration of biosorption observation of *A. granosa* and *M. nitidum* against Pb toxicity was 28 days. Wastewater (20 L/aquarium) and sediment (2 kg/aquarium) from the offshore oil drilling area were put in each of the twenty main treatment aquariums (each was 45×30×30 cm in volume). The analysis of lead level absorbed by the *A. granosa* shells and *M. nitidum* was conducted every 7 days. The analysis of dinoflagellate planktons was carried out every one other day, where 1 ml of sample from each aquarium was collected. The cells of the dinoflagellate were examined and counted with a hemocytometer and an optical microscope to determine the maximum growth of the species. Aeration was conducted by giving an O_2 blower to support the growth of *M. nitidum* seaweed.

Pb Level Absorbed in *Anadara granosa* Shells, *Monostroma nitidum*, Water, and Sediment

The shells of *A. granosa* and *M. nitidum* seaweed were dried in an oven at a temperature of 105 °C for 24 hours. They were then cooled in a desiccator. Two grams of sample were put in a Teflon bomb, and 1.5 ml of $HClO_4$ and 3.5 ml of HNO_3 were added. Then, it was closed and left untouched for 24 hours. After that, it was heated in a water bath at 60 °C for 2 hours until the solution was clear. Three ml of deionized double distilled water was added and the heating process was started again until the solution became dry. After cooling at room temperature, 1 ml of thick HNO_3 and 9 ml of deionized double distilled water were added, followed by the measurement of the heavy metal level through AAS using air-acetylene flame.

The analysis of Pb level in the water was done by putting 250 ml of the water sample in a Teflon separating funnel, which was then extracted with APDC/NaDDC/MIBK. The organic phase was extracted again with HNO_3 (Martin and Meybeck, 1979). The sediment sample was put in a Teflon beaker and dried in an oven at a temperature of 105 °C for 8 hours. After being dried, it was washed three times with distilled water. Then, it was dried again and crushed until it became homogenous. Five grams of sediment sample was then destructed in a Teflon beaker with HNO_3/HCl at a temperature of ± 100 °C for 8 hours (Loring and Rantala, 1992). Afterwards, the level of Pb in the water and sediment samples was determined with AAS using air-acetylene flame.

Algicidal Test of *Monostroma nitidum* on *Chattonella marina*'s Survivability

The species of *C. marina* was cultured aseptically in f/2 media (Gullard's, Sigma) at a temperature of 20 °C, lamination of 40 μmol/m^2/s using 40-watt FL40SD (Toshiba) fluorescent lamps with a lighting cycle of 12 hours of light: 12 hours of darkness. It was then sub cultured for 30 days. Before co-culture examination was conducted, *C. marina* species ere cultured for 7 days. *M. nitidum* thallus which was able to be collected was dried for 1 day at room temperature and was then blended until it became a dry material. The formation of rough extracts using methanol solution and water followed the method conducted by Jin (1997). 0.1 g of *M. nitidum* dry material was incubated with 5 ml of methanol 99.8 + % GC (Wako Pure Chemical) for 24 hours at room temperature. This methanol extraction was repeated 3 times and compiled. After that, the methanol soluble fraction was concentrated with a TC 8 (Taitec) concentrator.

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After the methanol evaporation of the *M. nitidum* seaweed extract residues was finished, a water-soluble fraction was added. 30 minutes before the algicidal test, all the extracts were centrifuged with 5417R (Eppendorf) centrifuge at the speed of 10,000 rpm at 4 °C.

To test the effect of *M. nitidum* methanol rough extract on *C. marina*, several *M. nitidum* seaweed extracts were used with methanol and water cultured by adding 1 ml of C. marina culture (cell density of 3 x 10^4 cell/ml) to produce final concentrations of 200 and 100 mg/L of methanol extracts and 800 and 400 mg/L of water extracts. They were then put in 24 well microplates (Iwaki) for the algicidal activity test. The observation was carried out for 4 hours. The cell survivability and mortality were measured and observed with a microscope. All the treatments in this research were carried out separately and repeated at least three times in an aseptic condition in all stages. The algicidal activities were counted using the formula of the number of dead cells / (the number of dead cells + the number of living cells) × 100%.

Quantification Analysis of Algicidal Substances of HDTA, ALA, and ODTA from *Monostroma nitidum* in a Water Medium

The samples of *M. nitidum* seaweed were dried for at least 24 hours at room temperature and processed to be dry powder by using a blender. The co-culture of shells, dinoflagellate, and selected seaweed dry powder (1.5 g/l, size 150 µm, autoclaved and non-autoclaved conditions) were mixed with ASW and stirred for 6, 12, 24, 36, and 48 hours in a dark condition and at a room temperature. The dry powder was then filtered with filter paper no. 2. The extract solution produced from this process was then changed to become fatty acid methyl esters using 2.5 ml of 3% HCl/MeOH and incubated for 1 day. After being concentrated in an evaporator, the residue was added with CH_2Cl_2.

The CH_2Cl_2 layer was separated with a sonicator and water content was eliminated with Na_2SO_4. Filtration was then conducted through a short column by using silica gel (62-230 µm, 500 mg) and CH_2Cl_2 solvent. The formed methyl ester was solved in hexane. The analysis of fatty acid methyl esters was carried out by using chromatography gas of GC-2014 which was equipped with a CP-Sil 88 capillary column for FAME fused silica WCOT, 50 m x 0.25 mm i.d, and 0.2 µm film thickness. It was then standardized with combined fatty acid methyl esters of C8-C22 and C14-C22. An injector, initial column, and final temperatures were at 300 °C, 170 °C, and 230 °C. The program rate was 5 °C/minute, while the initial column and final time were formatted for 15 and 5 minutes. Nitrogen was used as the carrier gas and the detector was carried out with a Flame Ionization Detector (FID).

Data Analysis

The data analysis used is experimental research which is included in inductive statistics by drawing conclusions and making decisions based on facts related to the content of the heavy metal Pb in shell, seaweed, water and sediment so that conclusions can be drawn from certain conditions based on some data (samples). In the research carried out also uses a test procedure (Pb level absorbed, algicidal test, and quantification analysis of algicidal substances) which will later refer to the use of assumptions about the distribution of sample data that does not need to be normally distributed.

RESULTS AND DISCUSSION

Fragment Profile Extract Protein of *Brachionus* sp.

Pb biosorption of *A. granosa* shells on Day 28 showed that the absorption of 36.95% of the total amount of Pb waste came from wastewater and sediment (5.57 and 0.004 mg/kg). For *M. nitidum* seaweed, the Pb absorption was 8.79% of the initial total amount of Pb waste from wastewater and sediment. The analysis of

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Pb levels during the research showed an interesting trend in which the levels of Pb in wastewater and sediment kept decreasing, but the biosorption of the shells and *M. nitidum* seaweed kept increasing (Table 1).

Table 1. The analysis of Pb levels (mg/kg) in wastewater and sediment from the offshore oil drilling area, *Anadara granosa* shells, and *Monostroma nitidum* seaweed.

Source	Day				
	0	7	14	21	28
Wastewater	0.004±0.001	0.003±0.001	0.003±0.001	< 0.001	< 0.001
Sediment	5.57±1.58	4.19±0.74	2.93±0.86	1.29±0.16	0.94±0.09
Shells	0.13±0.07	0.51±0.08	1.62±0.13	1.98±0.21	2.04±0.27
M. nitidum	< 0.001	0.001±0.01	0.17±0.03	0.26±0.06	0.49±0.19

The results of the Pb level analysis indicated that the presence of *A. granosa* shells had a significant impact on Pb biosorption. O_2 turbulence stirred the sediment causing the exposed Pb to be absorbed by *A. granosa* shells.

The observation of wastewater showed an interesting result because, through the plankton identification, a type of *C. marina* Dinoflagellate which belong to the Harmful Algal Bloom (HAB) Group was found. However, the number of *C. marina* kept decreasing as the observation duration became longer (Table 2).

Table 2. The selection of algicidal activities (%) of the methanol extract solution and water solution of *Monostroma nitidum* against *Chattonella marina*, cultured for 4 hours. The data were the Mean ± SD of at least three independent tests.

Seaweed	Methanol Extract (mg/L)		Water Extract (mg/L)	
	200	100	800	400
M. nitidum	92.89±2.91	41.55±1.37	15.74±1.77	4.86±1.93

The *M. nitidum* seaweed methanol extracts played an important role in suppressing the growth of *C. marina* because the morphological form of C. marina cells experienced a stative condition and elongation, which eventually caused the cells to disintegrate (Figure 1).

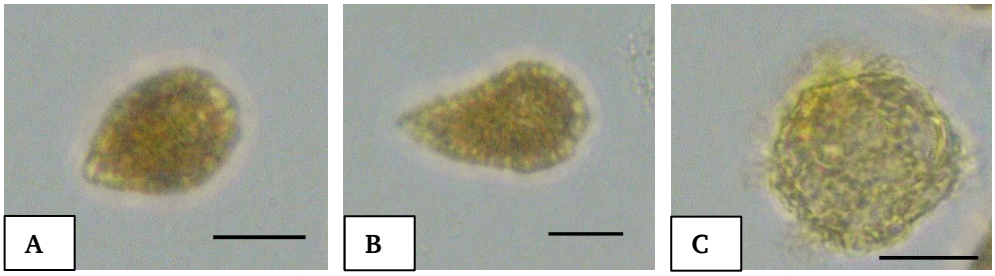

Figure 1. Algicidal activity from the seaweed methanol extract of *M. nitidum* against *C. marina*; A. the cell's original form; B. cell was motionless and had an elongated form; C. the cell was broken; the scale bar was 10 μm.

The biggest component of the polyunsaturated fatty acids (PUFA) of *M. nitidum* seaweed is 66% of the total fatty acids, which consist of hexadeca-4,7,10,13-tetraenoic acid, linoleic acid (HDTA), α-linolenic acid (ALA), and octadeca-6,9,12,15-tetraenoic acid (ODTA) which are very potent for the growth and survivability of dinoflagellate, amounting to 61.53 mg/100 g (Table 3).

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Table 3. Composition of fatty acids of *M. nitidum*.

Fatty Acids	Mean ± SD (% of total composition)
10:0	0.08±0.06
14:0	1.81±1.02
14:1, cis-9	0.88±0.29
16:0	18.28±6.62
16:4 (HDTA)	15.47±0.74
18:0	0.66±0.66
18:1, trans-9	3.45±0.78
18:1, cis-9	5.22±4.34
18:2,cis-9,12	4.19±0.47
18:3,cis-9,12,15(ALA)	24.69±1.32
18:4 (ODTA)	21.37±1.91
20:1	0.52±0.17
22:0	0.80±0.11
22:1, cis-13	5.27±3.72
PUFA:FA	0.66±0.08

PUFA: polyunsaturated fatty acid; FA: fatty acid.

The active material test of HDTA, ALA, and ODTA of the *dry powder M. nitidum* also showed the ability of the algicidal activity towards C. marina dinoflagellate and its active period in water. The analysis result showed that the active condition could be achieved up to 6 hours of incubation (Table 4).

Table 4. Active ingredients of HDTA, ALA dan ODTA released by *Monostroma nitidum* dry powder in sea water (mg/L) and algicidal activity (Means % ± SD) in *Chattonella marina* (cell density of 3 x 10^5 cell/ml).

Incubation Time (hour)	Dry powder Monostroma nitidum					
	Autoclaved			non-autoclaved		
	HDTA, ALA, ODTA (mg/L)	HDTA, ALA, ODTA (%)	AA (%)	HDTA, ALA, ODTA (mg/L)	HDTA, ALA, ODTA (%)	AA (%)
6	15.16	52.66	100	13.11	45.54	100
12	9.99	34.69	100	5.64	19.59	71.90
24	6.13	21.29	100	1.73	6.01	15.83
36	4.09	14.21	100	0.91	3.16	10.59
48	2.93	10.18	100	0.57	1.98	3.62

(%): % HDTA, ALA and ODTA released in sea water; AA: algicidal activity; 1.5 g/*l* of dry powder *Monostroma nitidum* in sea water containing 28.79 mg/*l* HDTA, ALA and ODTA.

Sediment from the offshore oil drilling area was identified to contain a higher level of Pb than that from the wastewater on Day 0. This was because the heavy metal dissolved in the water column and absorbed by the fine particles (suspended solid) would eventually settle in the bottom of the water. The accumulation of heavy metals in the sediment occurred relatively fast due to the water movement and tide patterns. Even though the early mechanism of heavy metals exposed in water will undergo a dilution process, due to gravitational force and water turbulence, the heavy metal compounds will accumulate in the sediment due to a settlement process. The accumulation of Pb in water sediment causes the sediment to contaminate the stability of nutrients which actually can be absorbed by other organisms (Tangahu *et al*., 2011).

The same thing will happen to demersal biota having filter feeder capability, which will be marred by higher heavy metal levels than pelagic biota. Heavy

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metals that are exposed to a water environment take the forms of free ions, organic ion pairs, and complex ions (Giraldo and Moreno-Piraján, 2013). However, in general, lead exposure in water is in the form of ions with an oxidation number of Pb^{2+} produced by industries, mining, and offshore oil drilling activities. Lead originating from leaded fuel is the source of pollution in the atmosphere and land which eventually ends in waters (Loring and Rantala, 1992).

Water quality also affects the heavy metal concentration in water areas, such as pH, salinity, temperature, and DO. Solubility of heavy metals is higher in low pH, which causes higher heavy metal toxicity (Jaishankar *et al*., 2014). The decrease in pH levels is caused by the decomposition process and the occurrence of both industrial and household wastes that disrupt the balance of the water ecosystem. The lowering salinity will also cause rising toxicity of heavy metals and higher accumulation of heavy metals. The increasing temperature of water areas also causes heavy metals to dissolve more. Lower DO levels will increase the toxicity of heavy metals in water areas as well.

Efforts to reduce Pb pollution have been conducted numerous times through chemical processes, such as the addition of chemical compounds to separate heavy metal ions or ion exchange resins, and other methods using active carbons, electro dialysis, and reverse osmosis (Wimalawansa, 2013). Nonetheless, these methods tend to create problems concerning the accumulation of Pb in sediment and water biota. The same thing happened with the use of microorganism biological agents, such as the process of bioaccumulation, bio remediation, and bio removal.

The most common obstacle is that the absorption of Pb takes longer time due to the limited capability of the organisms in doing an active uptake process simultaneously along with the consumption of Pb for the growth of the microorganisms and/or the heavy metal intracellular accumulation, which heavily depends on water quality, good pH, salinity, temperature, and DO. Similarly, in the passive intake process, Pb ions are attached to the walls of biosorbent cells, both through the exchange of ions on the cell walls with the heavy metal ions and through the formation of complex compounds between the heavy metal ions and functional groups like carbonyl, amino, thiol, hydroxy, phosphate, and carboxyl-hydroxy in a back-and-forth and fast way (Milojković *et al*., 2016). The presence of *A. granosa* shells dissolved in acid pH in a water area can affect the Pb level in that area due to the $CaCO_3$ content of the *A. granosa* shells that can cause a chemical reaction where the Ca atoms will be released easily and bind with heavy metals to form complex bonds (Budin *et al.*, 2014). As with *M. nitidum* seaweed, it will absorb the heavy metals with a passive uptake process (Abdel -Aty *et al*., 2013).

Shells are water organisms from the family of Pelecypods (Class Mollusca) that have a filter feeder characteristic. (Abdel -Aty *et al*., 2013) reported that the levels of heavy metals in shells depend on the shells' anthropogenic provenance, such as in the Gulf of Gdask which was recorded to be more than 20 μg/g, in Dutch Estuaries recorded to be more than 31.5 μg/g, and in Septiba Bay (Rio de Janeiro) which was recorded to exceed 13.1 μg/g. Until now, shells have been one the most favorite seafood dishes. However, there have been many cases of food poisoning due to shell consumption; a great number of which resulted in deaths. In general, poisonous shells and non-poisonous shells cannot be easily differentiated from their appearance. The poisons are likely to derive from the absorption of heavy metals from polluted waters and bioactive absorption by dinoflagellate planktons (Kleypas and Wilson, 2019). The use of shells due to their ability to bind heavy metals is an effort to improve the quality of water areas by reducing the levels of heavy metal pollution and maintaining the balance condition (homeostasis) and carrying capacity of the waters.

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The decrease in the cell density of *C. marina* dinoflagellate is likely due to the bioactivity of PUFA discharged by *M. nitidum*. Alamsjah *et al*. (2006) mentioned that the Chlorophyceae active content of PUFA in the forms of hexadeca-4,7,10,13-tetraenoic acid, linoleic acid, α-linolenic acid, and octadeca-6,9,12,15-tetraenoic acid was very potent to affect the growth and survivability of dinoflagellate. The isolation and structure determination of algicidal compounds showed that PUFA as HDTA, ALA, and ODTA were lethal for HAB species (McCracken *et al*., 1980; Chiang *et al*., 2004, Alamsjah *et al*., 2005). This is also supported by Oda *et al*. (1992) who stated that polyunsaturated fatty acids exposed in water areas (among others are active materials released by water organisms) will cause oxidation that will damage the integrity of Dinoflagellates' membranes.

Alamsjah *et al*. (2005) stated that the amphipathic nature of the double-binding chemical structure of polyunsaturated fatty acids had a tremendous biological effect on the ability of dinoflagellate to maintain its cell membranes. Kremer (1980) explained that macroalgae have significant differences in the composition of fatty acids between the individual classes of marine algae, marine and freshwater algae, and algae (in general) and terrestrial plants. In the present study, the amounts of HDTA, ALA, and ODTA, and algicidal activities of *M. nitidum* were higher than those of other macroalgae (Alamsjah *et al*., 2007).

The fatty acid composition profiles of each macroalga of Chlorophyceae were almost similar, with these marine green algae having a high concentration of C16 and C18 polyunsaturated fatty acids. These data are in agreement with the conclusions of other studies that the dominance of C16 and C18 PUFA is characteristic of green macroalgae (Jamieson and Reid, 1972). In this study, PUFA: FA ratio of *M. nitidum* showed high growth rates (>60%), as (Alamsjah *et al*., 2007) suggested that PUFA: FA ratio could be a useful indicator of the physiological status of algae. For example, higher ratios mean favorable growth conditions and high growth rates. This is because PUFAs are usually stable major components of cell membranes while saturated fatty acids are environmentally-sensitive storage products (Phleger *et al*., 1997). Thus, the biosynthetic substances of *M. nitidum* species are promising for practical harmful algal bloom control.

The algicidal compounds (HDTA, ALA, and ODTA) from the autoclave of dry powder of *M. nitidum* were higher in seawater than in the non-autoclave of dry powder of *M. nitidum* in a time-dependent manner. It means that the decomposition of the algicidal activity of *M. nitidum* was caused by biological agents (e.g., bacteria) and chemical reactions. Similarly, the algicidal activity of the dry powder autoclave of *M. nitidum* was also higher than that of the non-autoclave of dry powder of *M. nitidum* in a time-dependent manner under controlled conditions in the laboratory.

Meanwhile, Ikawa (2004) mentioned that effective levels of soluble PUFAs in long-term low-level exposures under natural conditions can have effects that are only observed with higher levels of acute doses. It was probably because the fatty acids from fresh tissues of macroalgae in seawater were released in gradual concentrations. The toxicity of green algae *Haematococcus* was caused by linoleic and α-linolenic acids at 12.5 mg/l in the field under natural conditions.

CONCLUSION

The Pb levels from polluted areas from an offshore oil drilling area near Camplong Beach, Madura decreased with the absorption process that occurred in the shell and seaweed. This is indicated by an increase in Pb levels detected in the body shell and thallus through laboratory tests. Interestingly, *A. granosa* shells and *M. nitidum* seaweed around oil drilling areas can absorb the toxic substance Pb thereby suppressing the adverse effects on human

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health and the survival of other aquatic organisms.

The engineering of *A. granosa* shells and *M. nitidum* as biological filters for absorbing Pb and decreasing the number of dinoflagellate cells in wastewater and sediment in the offshore oil drilling area shows a significant result. The shells of *A. granosa* shells and *M. nitidum* can be inexpensive and environmentally friendly materials for reducing heavy metals. Furthermore, the ability of *M. nitidum* seaweed with its algicidal contents (HDTA, ALA, and ODTA) to suppress the survivability of harmful algal bloom species of *C. marina* has a great potential for preventing the blooming of dinoflagellate *C. marina* and reducing the exposed toxins.

CONFLICT OF INTEREST

The author declares there is no conflict of interest.

AUTHOR CONTRIBUTION

Mochammad Amin Alamsjah: author correspondence, principal researcher, collecting data, analysis, and writing of the manuscript.

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