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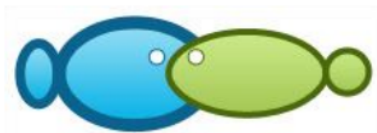
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Arsenic content, cell structure, and pigment of *Ulva* sp. from Totok Bay and Blongko waters, North Sulawesi, Indonesia

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Abstract. Small-scaled gold mining activities are abundant around Ratatotok area causing the condition of river to Totok Bay waters turn to brown color and have high sedimentation coming from the mining disposals. However, macro algae are still found in the area, one of which is *Ulva* sp.. This study was aimed at analyzing Arsenic (As) content in the seawater, sediment, and thallus of *Ulva* sp. collected from Totok Bay and Blongko waters, analyzing the pigment and the cell structure of the algae. The analysis of As followed APHA method; pigment analysis used HPLC following the effect of As on the cell structure used a Transmission Electron Microscope (TEM). As content in the seawater of Totok Bay and Blongko waters was < 0.0005 ppm, in the sediment it was 24 ppm for Totok Bay and 2 ppm for Blongko waters, in *Ulva* sp., it was 4.8 ppm for Totok Bay and < 0.5 ppm for Blongko waters. The cell wall of *Ulva* sp. from Totok Bay has been damaged, vacuola reduced, and the cell structure could hold heavy metal, while the cell structure of *Ulva* sp. from Blongko waters was not damaged and clearly showed the cell parts, such as cell wall and vacuole. The pigment types found in *Ulva* sp. from Totok Bay and Blongko waters were chlorophyll *a* and *b* and carotenoid.

Key Words: heavy metal, green algae, absorption, cell structure, pollution.

Introduction. Most public mining activities in Ratatotok district are concentrated in several villages, such as Soyowan, Ratatotok, and North Ratatotok. Number of gold miners and their activities are increasing reaching the residential areas. The materials, such as soil and stones are brought to the river margin to be grinded and mashed using river water. In this phase, cyanide is utilized and released into the river. The drain makes the river water in Ratatotok turn to brown and put sediment into the river flowing to Totok Bay (Mawikere et al 2015). Mercury (Hg) is often associated with other sulfide metal sediment, such as Au, Ag, Sb, As, Cu, Pb, and Zn, so that in the gold mineralization area, usually contains sufficiently high Hg and several other heavy metals (Herman 2006).

Totok Bay area is a mineralized area so that this area holds sufficiently high Hg content. Beside containing very high gold resource, there are other metals, such as As (BTKL-PPM 2005). Hg content in the sediment of Totok river from small-scaled gold mining using Hg is potential to have long-term environmental problems (Ministry of Environment 2004).

Previous studies show that metal concentrations in the seawater of Totok Bay range from 3.237-3.745 ppb for Hg, 0.24-0.35 ppb for As, respectively, while the sediment contains Hg of 0.10 ppm and As of 19.63-21.66 ppm (Umboh et al 2005). Heavy metal analyses in seawater of Totok Bay waters, according to Nasprianto (2017), found Hg of < 0.00005 ppm, while in Blongko waters there was Hg of < 0.00005 ppm. The heavy metal concentrations in the sediment of Totok Bay were 4.71 ppm for Cd, 10.7 ppm for Pb, 58 ppm for Zn, and 2.68 ppm for Hg, while those in Blongko waters were 0.03 ppm for Cd, 0.4 ppm for Pb, < 1 ppm for Zn, < 0.05 ppm for Hg, respectively. The heavy metal concentrations in *H. opuntia* collected from Totok Bay were 0.18 ppm for Cd,

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2.2 ppm for Pb, 5.10 ppm for Zn, and 0.74 ppm for Hg, while those of Blongko waters were 0.02 ppm for Cd, 0.2 ppm for Pb, < 0.5 ppm for Zn, and 0.009 ppm for Hg.

Although the concentration of heavy metals in water is relatively low compared to sediments, heavy metals will not be degraded. Heavy metal even could be absorbed and biologically accumulated in organisms, especially marine algae (Darmono 1995; Siahaan et al 2015). In Totok Bay, the green algae *Ulva* sp. is found year-round, plants up to 15 cm tall, light to bright green with small holdfast; thallus expanded into thin, glossy blades; margin undulating. This species is usually found at intertidal zone but may extend to subtidal zone, attached to rocks. This species is found in the bottom waters of the Totok Bay, always full of sediment. Algae have sufficiently high ability to absorb heavy metals because there are functional groups in the algae capable of binding with the metal ions (Putra et al 2003). These groups are carboxyl, hydroxyl, amine, sulfhydryl, imidazole, sulfate, and sulfonate in the cell wall of the cytoplasm (Bachtiar 2007). Impact of heavy metals on macro algae is to inhibit growth, for heavy metals could damage the chloroplast which is the most sensitive organ to heavy metals. Hence, it is necessary to analyze the effect of heavy metals on the pigment content and the cell structure of the algae. The objectives of the study were to analyze arsenic (As) concentration in seawater, sediment, and *Ulva* sp. in Totok Bay and Blongko waters, to examine the pigment content in *Ulva* sp., and to observe the cell structure of *Ulva* sp.

Material and Method

Sampling sites. Sampling sites were selected around Totok Bay waters, Minahasa Tenggara regency, and Blongko waters, Minahasa Selatan regency on May-July 2017. The former was used as control site (Figure 1). As concentration in the seawater, sediment, and *Ulva* sp. was analyzed with AAS (Atomic Absorbance Spectrophotometry) and pigment analysis in HPLC on September 2017, while the effect of heavy metals on the cell structure of the algae was assessed using Transmission Electron Microscope (TEM) in Eijkman Institute Laboratory Jakarta.

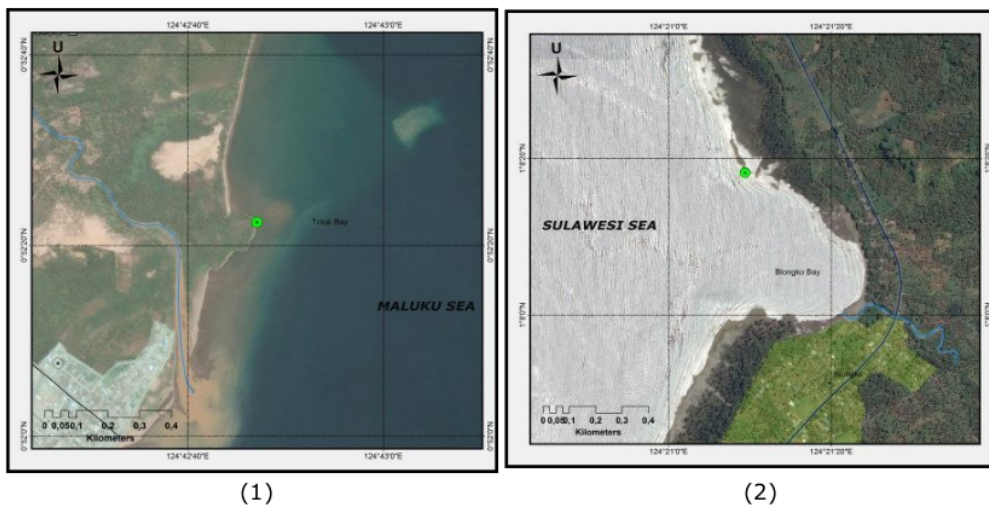


Figure 1. Map of sampling sites: (1) Totok Bay waters and (2) Blongko waters.

Research procedure. The analysis of As concentration followed APHA (2012) method, pigment of *Ulva* sp. used High Performance Liquid Chromatography (HPLC) based on Zapata et al (2000) method, the effect of heavy metals on the cell structure of *Ulva* sp. employed a TEM following the method of Eijkman Institute Laboratory.

Data analysis. Data of metal concentration, pigment, and cell structure were descriptively analyzed by comparing samples collected from Totok Bay and Blongko waters, while As content was compared with the quality standard established by Living Environmental Minister decree numbered 51/2004 for seawater, Canadian Council of Ministers of the Environment (CCME) for sediment, INS Number 7387-2009 for marine algae-National Standardization Agency (2009).

Results

Arsenic (As) concentration in seawater, sediment, and *Ulva sp.* As concentration in seawater of Totok Bay and Blongko waters was < 0.0005 ppm. Sediment of Totok Bay waters contained higher As concentration than that of Blongko waters, 24 ppm and 2 ppm, respectively. Similar condition occurred in *Ulva sp.* collected from Totok Bay waters, 4.8 ppm, and from Blongko waters, < 0.5 ppm (Figure 2).

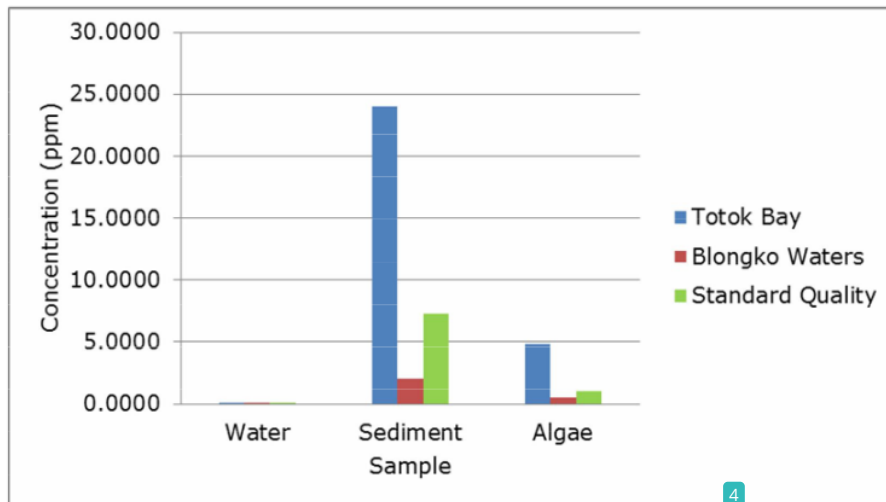


Figure 2. Arsenic concentration in the water, sediment, and *Ulva sp.* from Totok bay and Blongko waters.

As concentration in Totok Bay and Blongko waters was below the standard quality of Living Environmental Minister decree numbered 51/2004, 0.012 ppm. Low metal concentration in both locations could result from environmental factors, such as current and waves, besides the metal easily settles in the sediment. Heavy metals that enter the water will go through various processes, such as transport in tidal currents, dilution, associating with suspended materials, coagulation and falling to the bottom, associating with sediment organic matter, and absorbed by plankton (Siregar 2009).

As concentration in the sediment of Totok Bay is higher than the quality standard released by the Canadian Council of Ministers of the Environment (CCME), 7.24 ppm, while the sediment of Blongko waters was below the quality standard of CCME. In spite of that, the presence of heavy metals in the sediment could cause them be accumulated in the aquatic organisms and then in human body through the food chain. High sedimentation in Totok Bay from mining disposal activities is assumed as one of the causes of high metal concentration in the sediment. Leiwakabessy (2005) also reported that heavy metals easily bound organic matter, and sink to the sea bottom and unite with sediment so that the concentration in the sediment was higher than that in the water.

Moreover, As concentration in *Ulva sp.* collected from Totok Bay was higher than the quality standard of SNI No. 7387/2009, 1 ppm, but that in *Ulva sp.* from Blongko waters was below the quality standard. High As concentration in the sediment is directly proportional to the metal content in *Ulva sp.*, indicating the presence of metal absorption

process. Besides, metallothionein compound can bind with toxic metals, metallothionein being a cysteine-rich polypeptide capable of binding metals (Priyambada 2014). The ability of algae to absorb heavy metal ions is affected by several factors, such as seagrass species (Ryan et al 2012), morphology (Sawidis et al 2001), and time length of the seagrass exposure to the media (Tabaraki et al 2014).

Effect of metal on *Ulva sp.* cell structure. The cell structure of *Ulva sp.* collected from Totok Bay showed cell wall damage, reduced vacuole, and could hold heavy metals, while that of *Ulva sp.* from Blongko waters indicated no damage, and the cell parts, such as cell wall and vacuole, were clearly seen (Figures 3 and 4).

The cell wall around the chloroplast has been damaged. The cell wall of algae is highly susceptible to be damaged from heavy metals, since the heavy metals in the aquatic environment could be absorbed and bound on the cell wall of the algae (Figure 3).

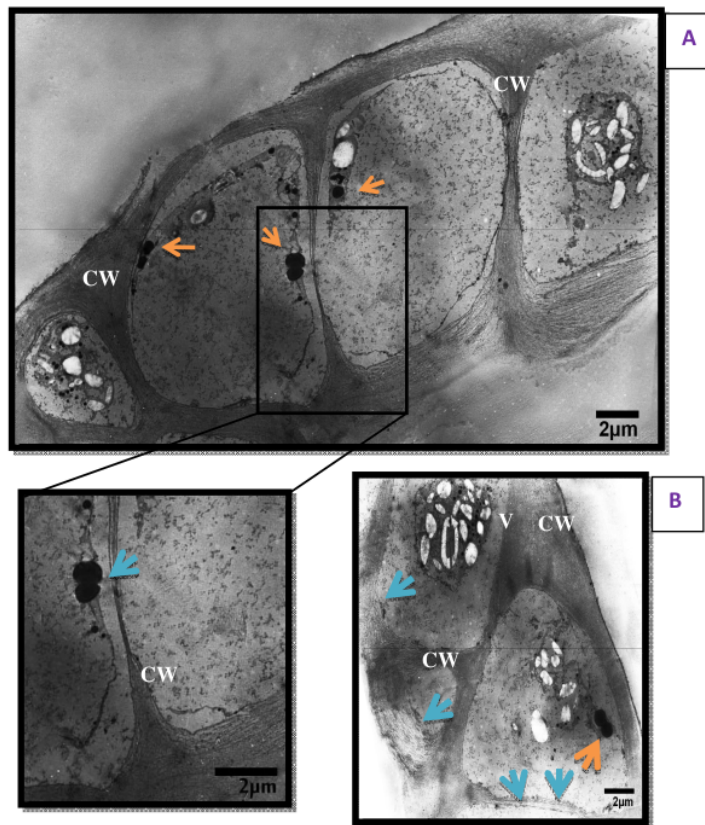


Figure 3. Cell structure of *Ulva sp.* from Totok bay; (CW) – cell wall, and (V) - vacuola (Transmission Electron Microscope, A: 1.200 enlargement and B: 3.000 enlargement) (private collection).

Figure 4 shows that the cell structure of *Ulva sp.* looks normal and no damage. In cell wall (CW), chloroplast (ch) and high number of vacuole (v) reflect that *Ulva sp.* actively photosynthesizes. Cell plasma and organelle are in good condition. Heavy metals were not found. Damage of cortex part will make the pollutant enter the cell faster, so that it could accelerate the damage of medulla cell. Cortex is part of the alga containing chlorophyll pigment for photosynthesis. With pigment presence, the alga can synthesize organic matters by absorbing sunlight as energy source (Tokida & Hirose 1975; Kimbal

1998). Change in cortex could result from loss of pigment in cell, where the accumulative heavy metal components enable them to build up in the cortex part, and inhibit photosynthesis, eventually causing the algae to lose the ability to grow.

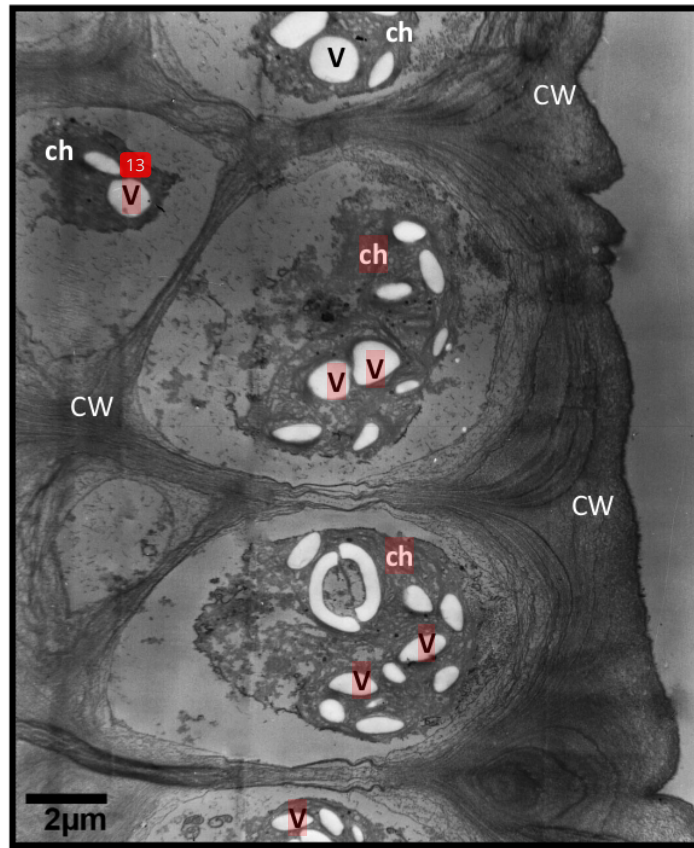


Figure 4. Cell structure of *Ulva* sp. from Totok bay waters; (CW) – cell wall, (ch) – chloroplast (ch), and (V) - vacuola (Transmission Electron Microscope, 3,000 enlargement) (personal collection).

Pigment content of *Ulva* sp. Pigment analysis in *Ulva* sp. collected from Totok Bay waters found chlorophyll *a* and *b* with carotenoid pigments, such as antheraxanthin and β -carotene. There were also found an unknown type of pigment with maximum absorption of 450 nm and resisting time of 18.007 min. In *Ulva* sp. from Blongko waters were found carotenoid pigment types as violaxanthin, antheraxanthin and β - carotene. This species had an identified pigment with maximum absorption of 438 nm and 465 nm with resisting time of 18.761 min (Table 1).

Number of identified pigments from Blongko waters is higher than that from Totok Bay. Based on the condition of Totok Bay waters with high enough metal concentration and damaged cell structure of *Ulva* sp. and TEM examination, there could be heavy metal contained in the cell structure, although *Ulva* sp. could still survive in Totok Bay waters. It could result from the presence of chlorophyll *a* and *b*, and carotenoid. Chlorophyll *a* is the main pigment responsible for photosynthesis. Thus, this pigment is important for the survivorship of the alga or competition with other organisms in a certain habitat (Gross 1991; Hegazi et al 1998; Pepe et al 2001). The presence of chlorophyll *a* is completed with its supporting pigment (chlorophyll *b*) and carotenoid (β -carotene, dinoxanthine,

lutein, siphonein) functioning to protect the chlorophyll *a* from photo-oxidation (Atmadja et al 1996; Green & Durnford 1996).

Table 1

Pigment types of *Ulva sp.* from Totok Bay and Blongko waters

Sample name	No	Pigment type	Absorption main peaks (nm)
<i>Ulva sp.</i> from Totok Bay waters	1	Unknown	450
	2	Antheraxanthin	447, 475
	3	chlorophyll <i>b</i>	460, 647
	4	chlorophyll <i>b</i>	460, 648
	5	Chlorophyll <i>a</i>	430, 662
	6	Chlorophyll <i>a</i>	430, 662
	7	β -carotene	455, 476
<i>Ulva sp.</i> from Blongko waters	1	Unknown	438, 465
	2	Violaxanthin	442, 470
	3	Antheraxanthin	447, 475
	4	Chlorophyll <i>b</i>	462, 648
	5	Chlorophyll <i>b</i>	462, 648
	6	Chlorophyll <i>a</i>	430, 663
	7	Chlorophyll <i>a</i>	430, 663
	8	β -carotene	455

Conclusions. As concentration in the seawater of Totok Bay and Blongko waters was < 0.0005 ppm. In the sediment, Totok Bay had much higher arsenic concentration than Blongko waters, 24 ppm and 2 ppm, respectively. Also, arsenic concentration in *Ulva sp.* from Totok Bay was higher than that from Blongko waters, 4.8 ppm and < 0.5 ppm. The cell structure of *Ulva sp.* from Totok Bay had started to damage on cell wall, reduced vacuole, and could possibly hold heavy metals, while that of *Ulva sp.* from Blongko waters was normal, and all cell parts, such as cell wall and vacuole, were clearly seen. The pigment types found in *Ulva sp.* from both study sites were chlorophyll *a*, *b*, and carotenoid that was assumed as one of the factors supporting *Ulva sp.* occurrence in Totok Bay waters. The results of this study show that green algae *Ulva sp.* is found in the Totok Bay throughout the year and it can be a phytoremediator.

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