Analysis of organochlorine insecticides in seaweed Kappaphycus alvarezii

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Analysis of organochlorine insecticides in seaweed *Kappaphycus alvarezii*

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Abstract. This study aimed to determine the reducing content and morphological changes of *Kappaphycus alvarezii* thallus in organochlorine (endosulfan) pollutant media. The main parameter in this study was the morphological structure of *K. alvarezii* thallus, while the supporting parameter in this study was the quality of seawater media. Based on Scanning Electron Microscope (SEM) analysis, *K. alvarezii* displayed changes in the morphological structure. Energy Dispersive Spectroscopy (EDS) analysis showed differences in shape: where the control showed shapes like lumps and the treatments looked like cubes. Based on the EDS analysis, control seaweed had the highest oxygen (O) content 43.29%, while the lowest element was titanium (Ti), 0.12%. Seaweed treated with 5 ppm of organochlorine pesticides was composed of several elements, with the highest being chlorine, 55.91% and the lowest calcium, 0.27%.

Key Words: endosulfan, pesticide, pollution, thallus.

Introduction. Organochlorines are highly soluble in fat and are absorbed in body tissues with high lipid content, such as the brain and liver. As a result, levels in the blood tend to be much lower than levels in adipose tissue. The lipophilic tendency of organochlorines causes excessive systemic effects in overdose (Li et al 2016).

K. alvarezii is a seaweed with high economic value that can increase the economic income of the community, especially of seaweed cultivators. Seaweed is one of the important fishery commodities that is widely used in various fields, both related to food and non-food industries. Seaweed produces colloidal compounds called phycocolloids, namely agar, alginate and carrageenan (Alba & Kontogiorgos 2019). Utilization of seaweed includes the food industry, cosmetics, pharmaceuticals, medicine, and other industries. Therefore, it is very necessary to analyse the effect of organochlorine insecticides on seaweed.

Sundhar et al (2019) found that total organochlorine pesticide residues in *Gracillaria acerosa* ranged from 6 ng g^{-1} to 508 ng g^{-1}, while for *Gracillaria verrucosa* it ranges from 0.36 ng g^{-1} to 270.41 ng g^{-1}. Maroli et al (1993) found that the concentrations of polychlorinated biphenyls (PCBs) and dichloro diphenyl trichloro ethane (DDTs) were about 1.5 times higher in the thallus in terms of the amount of absorption concentration. One type of organochlorine compound that is widely used by the community is endosulfan, because the price is accessible for everyone and it is effective in destroying nuisance insects. This pollutant can provide benefits for the agricultural sector. However, it can have a negative impact on life in the waters (Rompas 2010). The purpose of this study was to identify the effects of organochlorine pollutants on morphological changes of seaweed *K. alvarezii*.

Material and Method

Sampling sites. Sampling was carried out from April to July 2021 in Raprap Village, Gaze District, South Minahasa Regency, Indonesia. Station 1 represented a cultivation area where the samples were collected, station 2 was a fish culture area and station 3 was a river mouth (Figure 1).

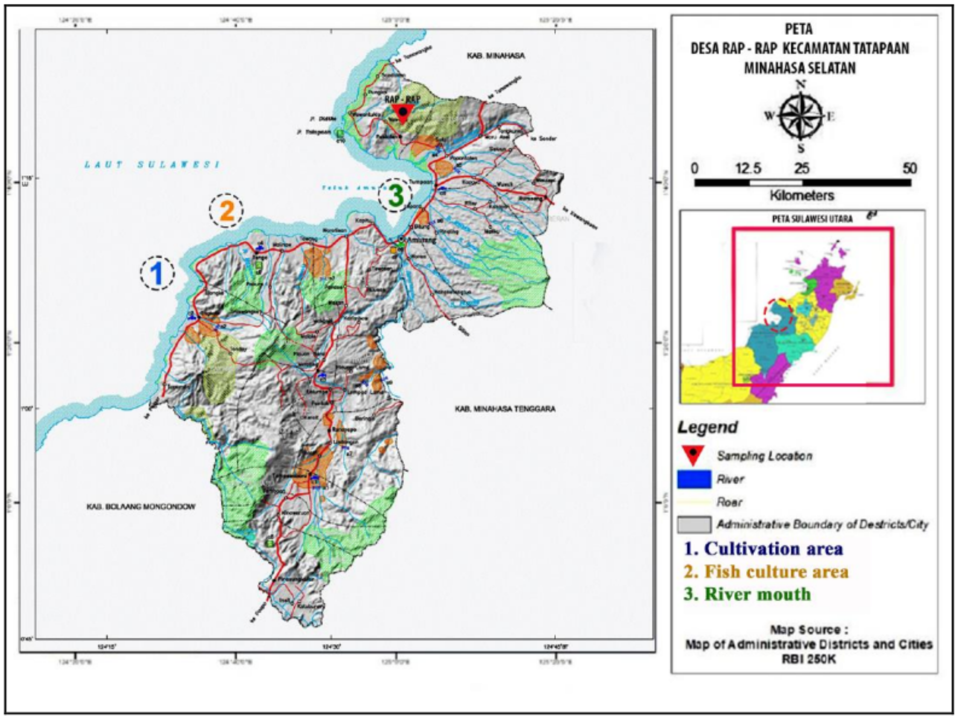

Figure 1. Map of the sampling site; station 1 - seaweed cultivation area; station 2 - fish cultivation area; station 3 - an area close to the river mouth.

Seaweed sampling. Seaweed (*K. alvarezii*) was collected in April 2021. This species is mostly cultivated by seaweed farmers in Rap-rap Village. Thallus of two weeks old seaweed were collected, placed into a coolbox and transported to the Aquaculture Technology Laboratory of the Faculty of Fisheries and Marine Science, Sam Ratulangi University, Manado, Indonesia.

Sample treatment. 500 g of seaweed samples were placed in aquaria containing sea water with a salinity of 32 ppt, a pH between 7.6 and 8, dissolved oxygen between 5.13 and 6.28 ppm. The seaweed were cultured in three replications, each being treated with 5 ppm organochlorine for one week. Observations were carried out on the changes in color and thallus. After exposure, the seaweed was cleaned with fresh water, soaked for one night and then dried at room temperature. The same process was conducted for the control.

Sample analysis. The exposed seaweed was placed in a 30 x 10 cm stainless tray and dried in an electric oven at a temperature of 60°C for 36 hours, then sent to the laboratory of the Faculty of Mathematics and Natural Sciences (MIPA), Bandung Institute of Technology, for Scanning Electron Microscope (SEM) analysis in order to observe the morphology, topography and structure of thallus tissue. Energy Dispersive X – Ray

Spectroscopy (EDS) was used to determine the elements contained in the sample and their distribution.

Results and Discussion. SEM analysis displayed the shape and size of nanoparticles contained in *K. alvarezii* (Figures 2 and 3). The 3000x magnification showed that the morphology of the *K. alvarezii* nanoparticles for the control had a unified morphological structure compared to that of seaweeds treated with organochlorine pesticides with the same magnification.

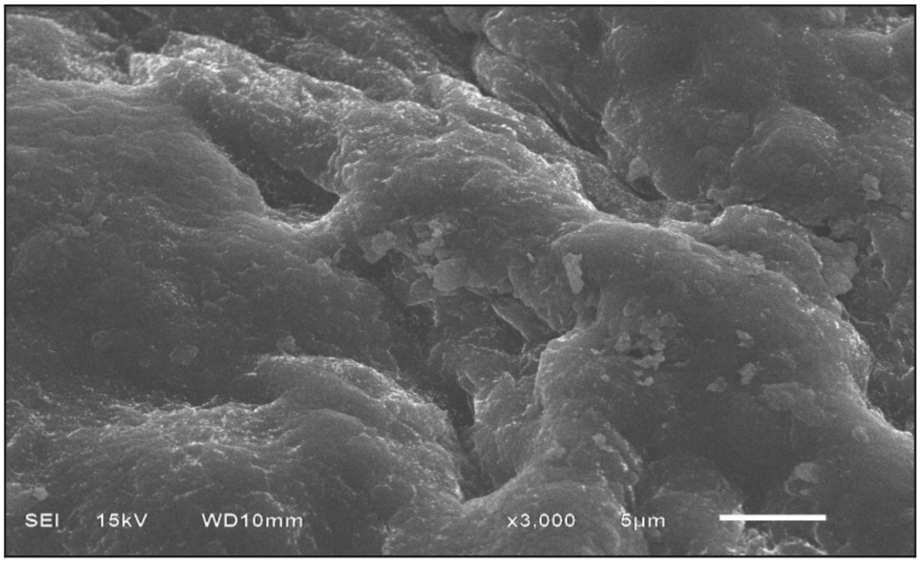

Figure 2. SEM analysis of control seaweed (3000x).

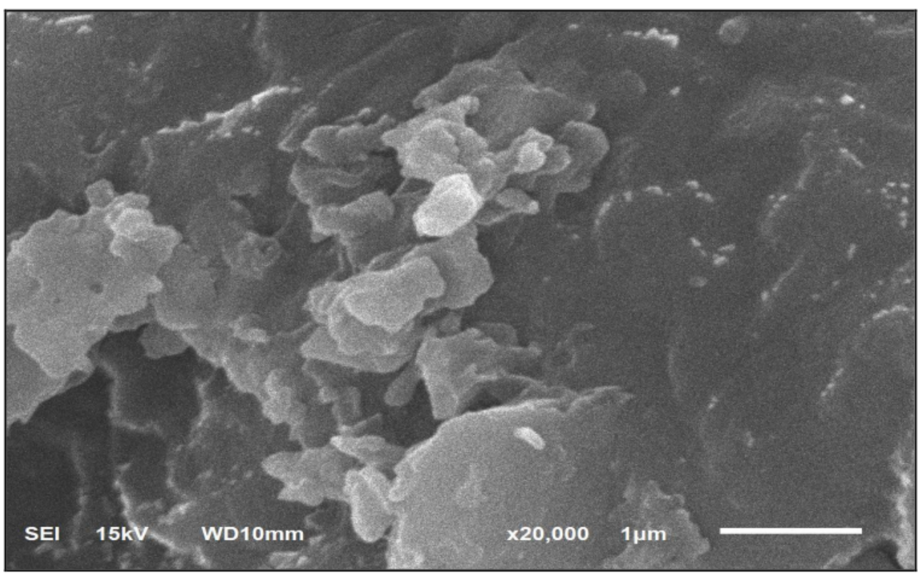

Figure 3. SEM analysis of control seaweed (20000x)

After observing the seaweed from control with 20000x magnification, the morphological structure presented clumps that coalesced, while seaweed treated with 5 ppm organochlorine pesticides clearly formed irregular boxes or cubes (Figures 4 and 5).

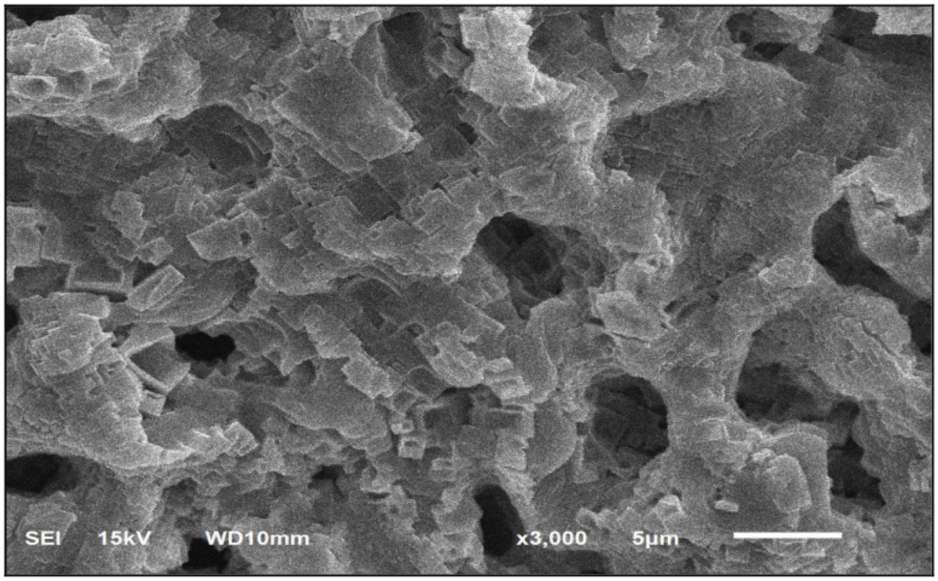

Figure 4. SEM analysis of organochlorine treated seaweed (3000x).

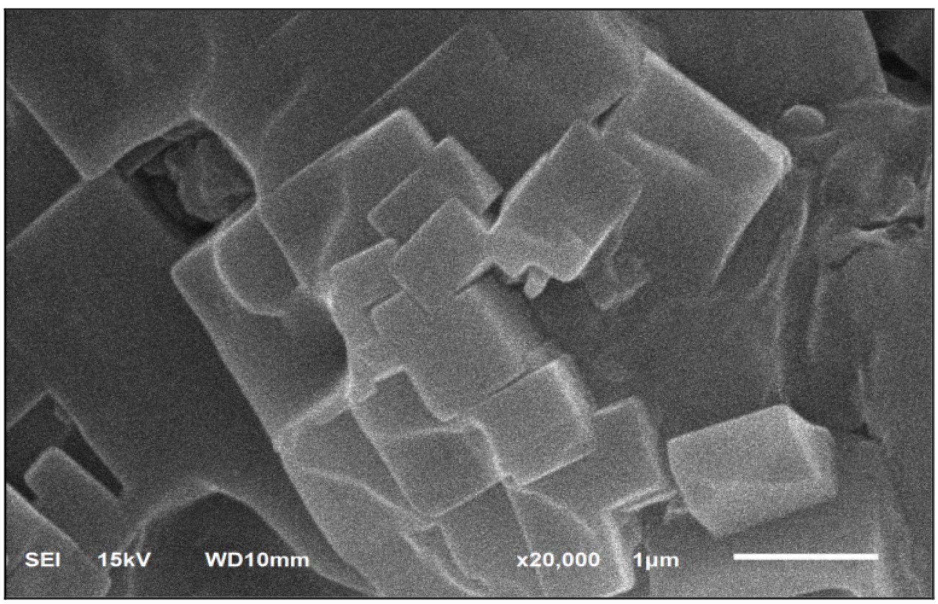

Figure 5. SEM analysis of organochlorine treated seaweed (20000x).

Based on the EDS analysis, control seaweed was composed of several elements in which the highest was O (oxygen) with a value of 43.29%, while the lowest element was Ti (Titanium), with 0.12 % (Figure 6; Table 1).

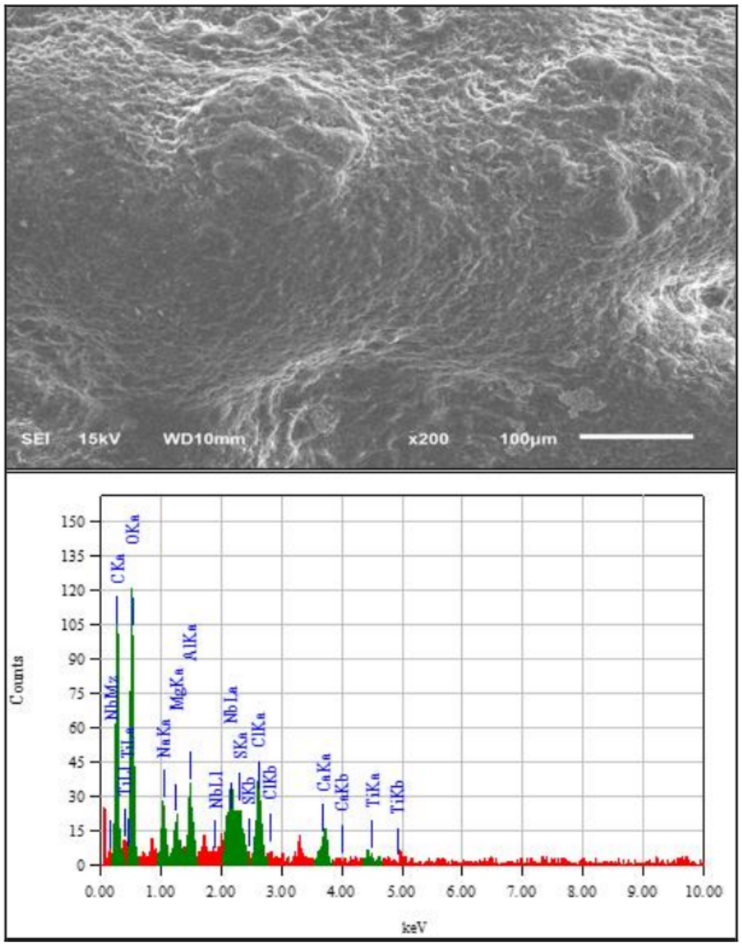

Figure 6. EDS analysis of seaweed not contaminated with organochlorine.

Table 1

ZAF Method Standardless Quantitative Analysis (Fitting Coefficient: 0.3805)

Element	*(keV)*	*Mass %*	*Error %*	*Atom %*	*Compound*	*Mass %*	*Cation*	*K*
C	0.277	47	0.06	58.31				31.9662
O	0.525	38.51	0.16	35.86				43.2915
Na	1.041	1.91	0.06	1.24				2.7107
Mg	1.253	0.81	0.06	0.5				1.0955
Al	1.486	1.97	0.06	1.09				3.0111
S	2.307	1.26	0.06	0.59				2.5644
Cl	2.621	2.76	0.07	1.16				5.4382
Ca	3.690	1.52	0.13	0.57				3.1229
Ti	4.508	0.08	0.18	0.02				0.1289
Nb	2.166	4.17	0.18	0.67				6.6707
Total		100.00		100.00				

Seaweed treated with 5 ppm organochlorine pesticides presented several elements, the highest being chlorine (55.91%) and the lowest was calcium (0.27%) (Figure 7; Table 2).

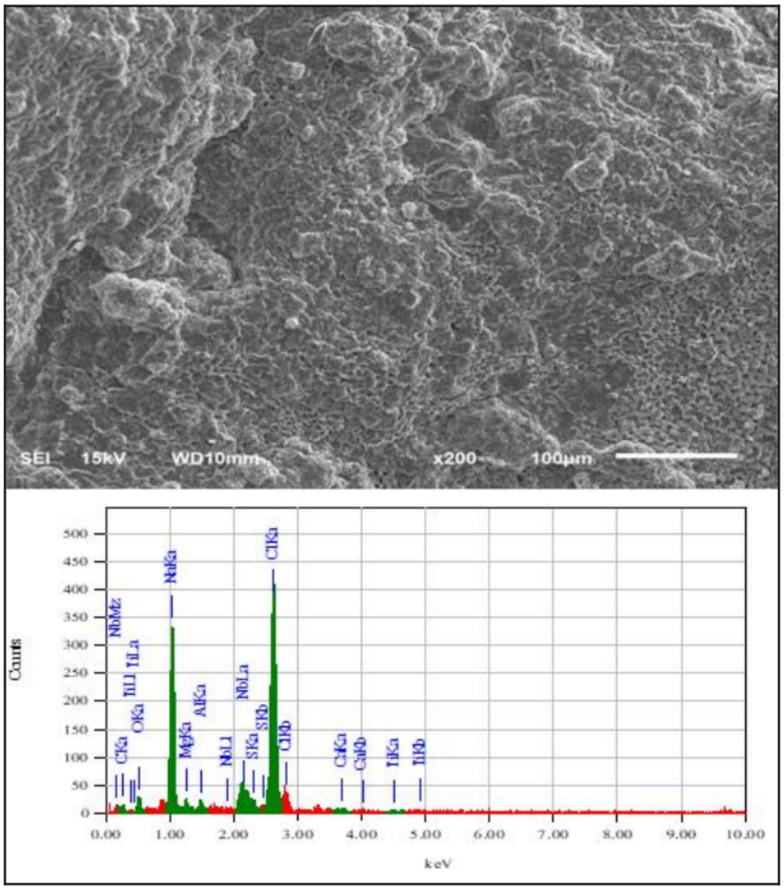

Figure 7. EDS analysis of seaweed contaminated with organochlorine.

Table 2

ZAF method standardless quantitative analysis (fitting coefficient: 0.2377)

Element	*(keV)*	*Mass %*	*Error %*	*Atom %*	*Compound*	*Mass %*	*Cation*	*K*
C	0.277	16.50	0.60	31.39				1.4043
O	0.525	9.88	0.31	14.11				6.5422
Na	1.041	23.69	0.09	23.55				28.3163
Mg	1.253	1.16	0.11	1.09				0.948
Al	1.486	1.14	0.11	0.96				1.0748
S	2.307	0.36	0.10	0.25				0.4868
Cl	2.621	42.31	0.12	27.28				55.9125
Ca	3.690	0.22	0.25	0.12				0.2723
Ti	4.508	0.29	0.33	0.14				0.3237
Nb	2.166	4.46	0.31	1.10				4.7191
Total		100.00		100.00				

Sundhar et al (2020) reported that the presence of aldrin residues in seaweed was not very significant, as the concentrations ranged from 0.022 to 2.42 ng g^{-1}. This value was below the Maximum Residue Limits (MRL) as much as 10 ng g^{-1} for aldrin derivatives in green leaf plants. Seaweed contains lipophilic compounds with low water solubility (0.027 mg L^{-1}) (Sundhar et al 2020; Sundhar et al 2019). Most of the residue is absorbed as aldrin by the seaweed. Aldrin derivatives were detected in low concentrations in October, 0.68 ng g^{-1} and 0.80 ng g^{-1}, whereas in November and December the concentrations were 0.56 ng g^{-1} and 3.97 ng g^{-1}, respectively (Sundhar et al 2020); Qiu et al 2017). In January, aldrin residues were slightly high, varying from 0.79 to 2.42 ng g^{-1} (Liu et al 2020). According to Sundhar et al (2020), *Ulva lactuca* accumulates mainly aldrin derivatives, but also appreciable amounts of dieldrin derivatives. It has been observed that more organochlorine pesticide (OCP) residue accumulation occurs after the rainy season, but this was not recorded previously. Addition of organochlorine pollutant (endosulfan) decreased agar content because organochlorine containing chlorine was chlorinated with H in the medium to form HCl. This HCl is corrosive, being able to weather the epidermal cells of the thallus of *Gracilaria verrucosa* (Riswanti et al 2013).

Qiu et al (2017) reported that polybrominated diphenyl ethers (PBDE) and OCP levels were higher in phytoplankton than in *Ulva* sp., indicating that phytoplankton with larger surface area had higher absorption efficiency for persistent organic pollutants (POPs) than *Ulva* sp. The bioconcentration factors (BCFs) of dichloro diphenyl trichlorethane (DDT) and PBDE in phytoplankton from two bays were in the range of 105-106, indicating that bioconcentration may be one of the main sources of POPs, and algae could be an important route for POPs to move towards higher trophic levels.

Tumembouw et al (2021) found that the type of organochlorine pollutant was aldrine in substrate in Megamas at 1.02 ppm, followed by the substrate in Tumumpa, with 0.925 ppm, and finally Malalayang, with 0.492 ppm.

Pavoni et al (2003) stated that in most of the seaweed samples in the Lagoon of Venice containing DDT in seaweed tissue, there were several types of DDT metabolites, namely DDD representing 51% of the total DDT related compounds, DDE with 34%, and DDT with 16%. The highest concentrations of pesticides were found in *Cystoseira* and *Fucus*, two species of Phaeophyceae characterized by perennial life cycles, and thus longer periods of exposure to contaminants.

Conclusions. The cell structure of *K. alvarezii* subjected to organochlorine in this study was damaged in the cell membrane, with many cells dying and being damaged compared to the control, where the cell structure appeared normal and there was no damage.

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Conflict of Interest. The authors declare that there is no conflict of interest.

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