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Short Communication

Organochlorine Exposure Influences the Cellular Morphology of Red Algae *Eucheuma denticulatum* (N.L. Burman) Collins & Hervey, 1917

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Abstract

Organochlorine compounds not only pollute marine waters but also interfere with the survival of marine biota. Organochlorine compounds absorbed by organisms disrupt metabolism and inhibit cellular functions. The implication of this research is to prevent and reduce the disposal of organochlorines into the environment because they can accumulate in soil, water, and air, remaining for years in the environment. This accumulation can affect food chains and negatively affect ecosystems and marine animals. This research aimed to investigate the impact of organochlorine content on the surface morphology and biomineral characteristics of the red alga E. denticulatum cells. Electron Microscope (SEM) analysis was used to observe particle morphology surfaces down to 1 nm, while Energy Dispersive Spectroscopy (EDS) was used to analyze the specimens' element composition and chemical characteristics. Energy Dispersive Spectroscopy (EDS) analysis revealed that red algae had the highest content of Chlorine (Cl) at 57.20%, followed by Sodium (Na) at 34.84%, Oxygen (O) at 5.21%, Calcium (Ca) at 1.64%, and the lowest element being Sulfur (S) at 1.11%. Overall, this research demonstrates the negative impact of organochlorine content on the morphological structure and biomineral composition of E. denticulatum, highlighting the need for effective measures to prevent and reduce organochlorine pollution in marine environments. Further research could focus on specific mechanisms of organochlorine toxicity and potential remediation strategies.

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1. Introduction

Organochlorine insecticides are persistent organic pollutants (POPs) that are difficult to degrade in nature, have slow biodegradation properties, and can move in biotic and abiotic environments (Burkow and Kallenborn, 2000). Insecticides will journey long before reaching coastal areas (Anasco *et al.*, 2010). Almost all insecticide residues can undergo physicochemical and biological decomposition during transport into the marine environment. Increases in pollutant concentrations are generally a consequence of anthropogenic waste disposal in liquid form, urban waste disposal, and agricultural and industrial runoff. Organochlorine insecticides are hydrocarbon compounds whose residues can last for decades in the environment, eventually ending up in seawater with the flow of rain (Aguayo-Quiroz *et al.*, 2020).

The organochlorine insecticide is generally used to protect vegetable crops, cotton, and tobacco and may be transported to the marine environment through runoff discharge (Ebenezer and Ki, 2014). Organochlorine insecticides are hydrocarbon compounds whose residues can last for decades in the environment until they eventually end up in the sea along with rain (Handini *et al*., 2013). Organochlorine compounds are not easily degraded; thus, they pollute marine waters and interfere with marine biota's survival (Jayaraj *et al.*, 2016). The decay of organochlorine compounds absorbed by organisms disrupts metabolism and inhibits cellular functions (Vagi *et al.*, 2021; Perez *et al.*, 2021; Nunes, 2022). DDT-type organochlorines have been banned in Japan for the past 25 years, in Europe for the past 15 years, and most recently in the United States for the past two years (Hu *et al.*, 2009). It has been banned by the Indonesian government since 1973, according to instructions from the Minister of Internal Affairs State, Minister of Health, and Minister of Agriculture of the Republic of Indonesia No: 33 of 1983 concerning the supervision of the use of DDT pesticides. In Indonesia, DDT was used in case of a dengue fever outbreak caused by mosquitoes (Gaston, 1994; Hu *et al.*, 2009). A report by (Sundhar *et al.*, 2020) showed that the Brown alga *Sargassum wightii* accumulated more OCP than *Padina tetrastromatica, Ulva lactuca, Caulerpa racemosa,* and *Gracilaria edulis.* The ability of seaweeds to accumulate pesticides was found to be species-specific and not class-specific.

Seaweeds are used for a wide range of products, from food to bioactive compounds for medicine. The production of world aquaculture seaweed has increased markedly, nearly tripling between 2000 and 2014, from 9.3 to nearly 27 million tonnes (Chung *et al.*, 2017). The red alga *Euchema denticulatum* is a commercial species that produces carrageenan and is used in food, cosmetics, pharmaceuticals, and other industries. However, there is still relatively little information on the specific impact of organochlorines on the coastal and marine environment. Moreover, the impact on the thickness of cellulose and other elements, such as chlorine (Cl), oxygen (O), sodium (Na), and others on algae, has never been studied. The morphology of the algae itself influences the quality of red algae. Dissolved organochlorines can damage the cell structure and thickness of seaweed cellulose, characterized by a color change from usual to slightly pale, reducing the quality of algae. In North Sulawesi, organochlorine insecticides are widely used in agricultural, animal husbandry, industrial, and domestic activities. If this waste is not managed correctly, it will flow into seawaters through rivers, polluting the environment and harming marine organisms, including algae.

The concentration of organochlorine in the sea that is safe for *Eucheuma* is unknown. To the best of our knowledge, there has been no research on the effects of organochlorines on marine algae. This research aimed to evaluate the effect of organochlorine concentration on the morphological surface and biomineral characteristics of the cell of *E. denticulatum*. This research is essential to prevent and reduce the disposal of organochlorines into the environment because they can accumulate in soil, water, and air and remain for years in the environment, accumulating in food chains and negatively affecting ecosystems and marine animals.

The implications of studying organochlorines for environmental impact are significant, as organochlorines are notorious for their persistence in the environment. They can accumulate in soil, water, and air, and their slow degradation means they can remain in the environment for years. This persistence can lead to the accumulation of these compounds in food chains, which may negatively affect ecosystems and wildlife.

2. Materials and Methods

This research occurred from January 20, 2021, to June 20, 2022, at the Faculty of Fisheries and Marine Sciences, Sam Ratulangi University, Manado. The effect of organochlorine on cellulose thickness was then analyzed using IBM-NCSS (*Number Crunch Statistical System*).

2.1 Materials

2.1.1 The equipments

The equipment used in this research includes: Spectrophotometer, cool box, glass container, beaker glass, aerator, scales, and Water quality checker Horiba U-50-10.

2.1.2 The materials

The materials used in this study includes: ultraviolet lamps, distilled water, alcohol, IDDT, DDT, Endrin, Eldrin, red algae, sea water, and gallons of water.

2.1.3 Ethical approval

This study does not require ethical approval because it does not use experimental animals.

2.1.4 Sample collection

The samples were collected from seaweed farming on Nain Island (Figure 1), promptly placed in a cool box, transported to the laboratory, and cultured in 15 aquaria, each measuring 60 x 40 x 40 cm. A stock organochlorine solution of 100 ppm was prepared in a separate aquarium, which was then diluted to obtain concentrations of 1 ppm, 5 ppm, 10 ppm, and 20 ppm by taking 50 mL, 100 mL, 500 mL, and 1000 mL, respectively. The control treatment used a separate aquarium without the addition of organochlorine.

2.1.5 Sample preparation and organochlorine exposure

500 g of algae samples were cultivated in each aquarium and then exposed to organochlorine as treatment. After one week of exposure, the algae samples were removed from the aquarium, washed with fresh water, soaked overnight, dried, and ground to a powder.

2.2 Methods

2.2.1 Sample identifications

Scanning Electron Microscope (SEM) analysis was employed to observe the particle morphology

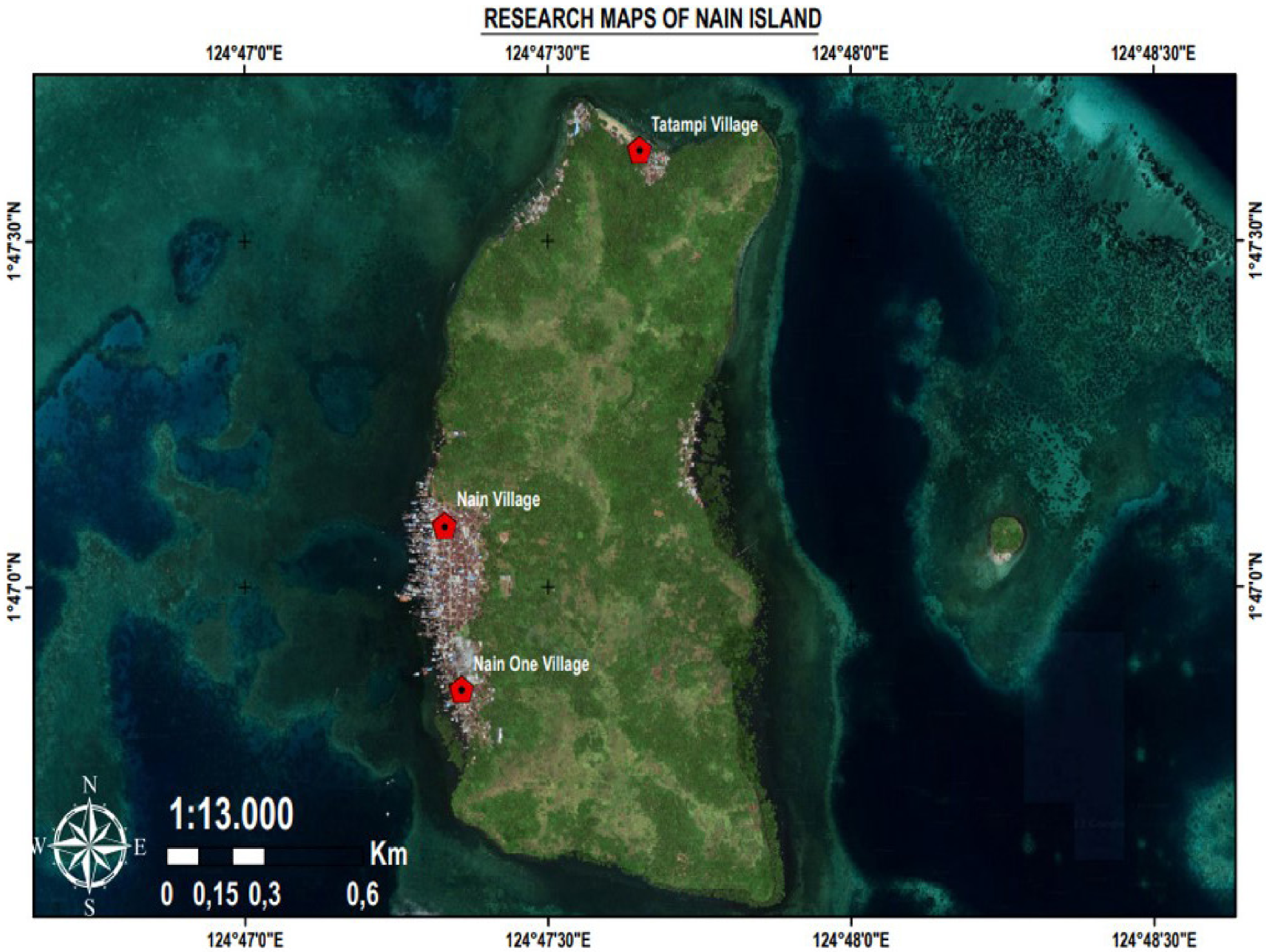

Figure 1. Map of research location.

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surface up to 1 nm (Charurvedi and Dave, 2012), while the element composition and chemical characteristics of the specimen were analyzed using Energy Dispersion Spectroscopy (EDS). The analysis was conducted at the Laboratory of the Basic Science Centre, Bandung Institute of Technology. Morphological characteristics, crystal structure, and elemental information of the specimen were analyzed using Transmission Electron Microscopy (TEM) (Tang and Yang, 2017).

2.3 Analysis Data

The data were analysed descriptively using figures and tables, while the effect of organochlorine on cellulose thickness and mineral content was analysed through ANOVA at a confidence level of 0.05.

3. Results and Discussion

3.1 Results

3.1.1 Images analysis

SEM analysis displayed images of cell structures in terms of cellulose fiber flakes taken at 20,000 magnifications. Using the ImageJ application, the thickness of cellulose fiber varied from 0.858 to 2.271 µm. The average thickness of cellulose was significantly different between the control (a=1.19 µm) and treatments at 1 ppm (b=1.92 µm), between 5 ppm (c=0.92 µm) and 10 ppm (d=1.57 µm), as well as between 10 ppm and 20 ppm (e=0.91 µm). The thickness of cellulose in the control treatment was relatively similar to that at 20 ppm, and at 1 ppm and 10 ppm, it was relatively the same (Figure 2).

The results showed that organochlorine significantly affected ($p<0.01$) the cellulose thickness of *E. denticulatum* (Table 1). The mean cellulose thickness of the control treatment (a=1.19 µm) was significantly different ($p<0.05$) compared to the 1 ppm treatment (b=1.92 µm) and 5 ppm treatment (c=0.92 µm) but not different compared to the 20 ppm treatment (0.91 µm). The cellulose thickness of the 5 ppm treatment was significantly different compared to the 10 ppm treatment (d=1.57 µm) but not different compared to the 20 ppm treatment (e=0.91 µm). The treatment at 1 ppm was not different compared to the 5 ppm and 10 ppm treatments, while the treatment at 5 ppm was not different compared to the 20 ppm treatment (Figure 3).

3.1.2 Biomineral content

The detection results of the red algae flour sample at 500x magnification reveal the elemental content of the sample. EDS detected several elements, with the dominant percentage being the Cl element (57.20%). This indicates the ion transfer of chlorine content in red algae cells, both from inside and outside, as the Cl element is an ion that can easily absorb electrons (Arias and Botte, 2020). SEM analysis results by Singkoh *et al.* (2019) showed lower Cl elements (17.38%), while research by Kepel *et al.* (2021) reported the lowest Cl content (4.91%). Overall, the

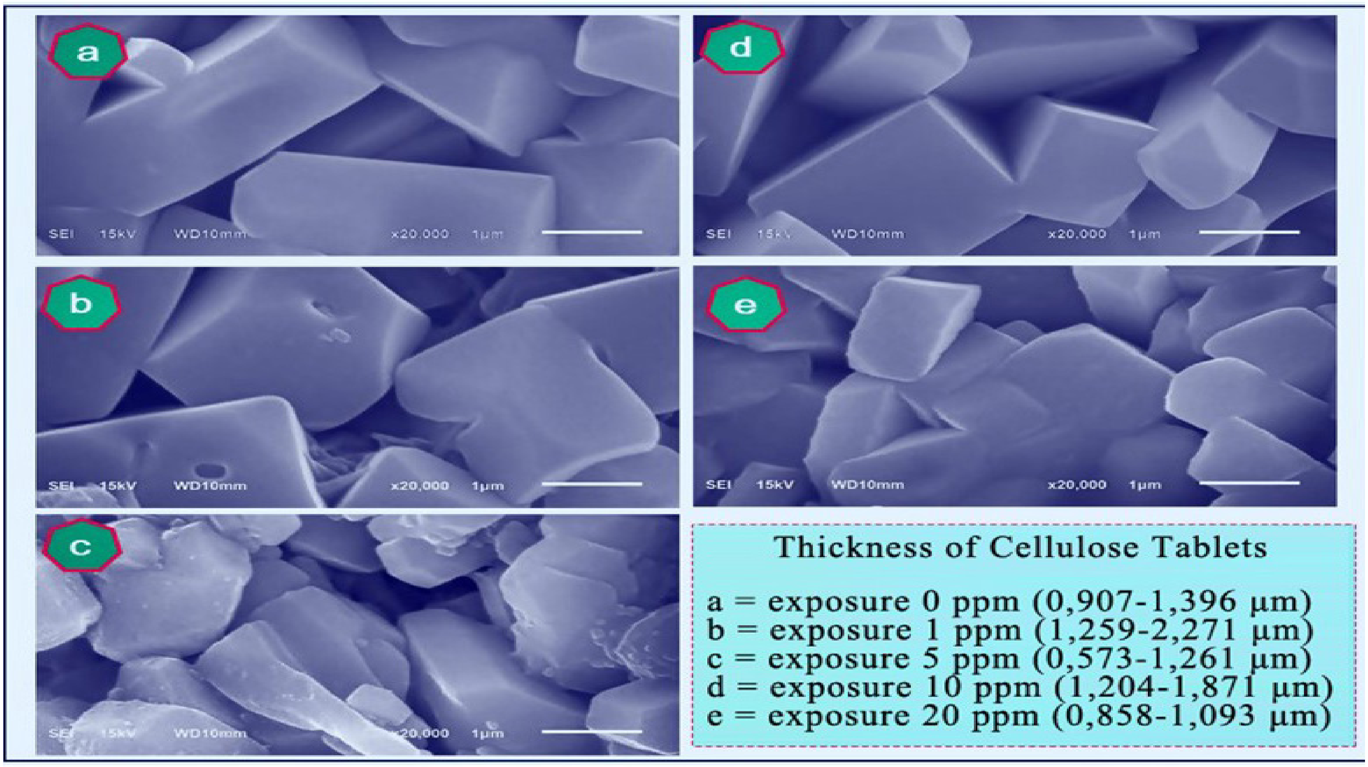
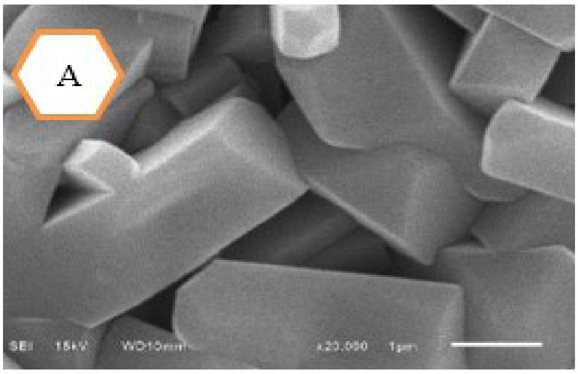


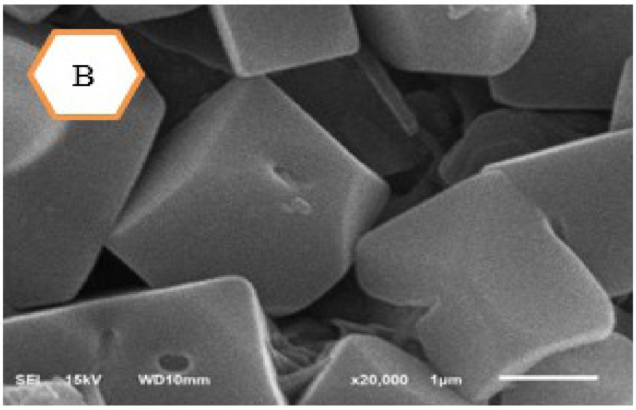
Figure 2. The thickness of *E. denticulatum* cellulose tablets.

Table 1. ANOVA of the organochlorine effect on the tablet thickness of the red algae cellulose.

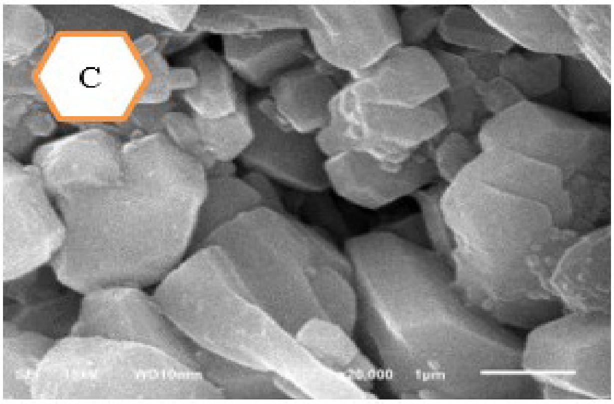
Tablet Thickness	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.903	4	.976	11.535	.000
Within Groups	1.692	20	.085		
Total	5.595	24			



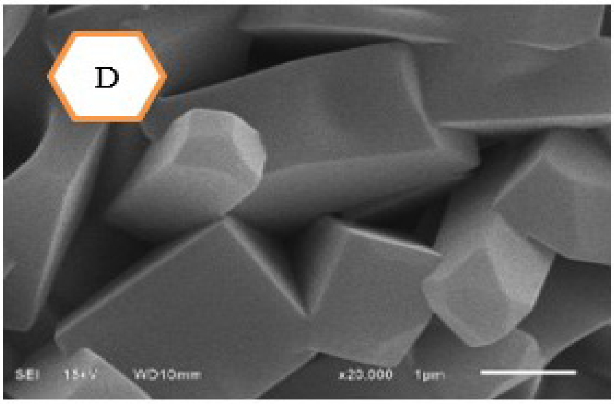
SEM-red alga at control, 20000x



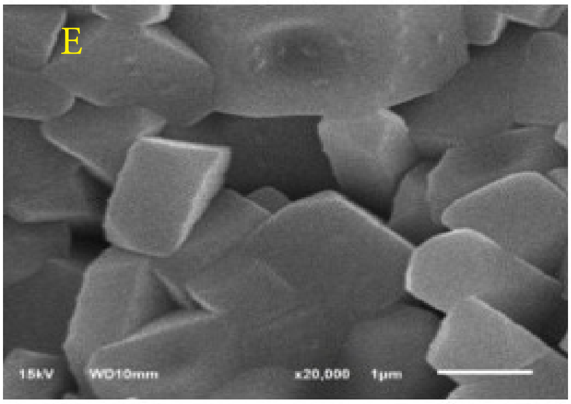
SEM-red alga at 1 ppm, 20000x



SEM-red alga at 5 ppm, 20000x



SEM-red alga at 10 ppm, 20000x



SEM-red alga at 20 ppm, 20000x

Figure 3. SEM analysis of *E. denticulatum* (A: control, B: 1 ppm, C: 5 ppm, D: 10 ppm, E: 20 ppm).

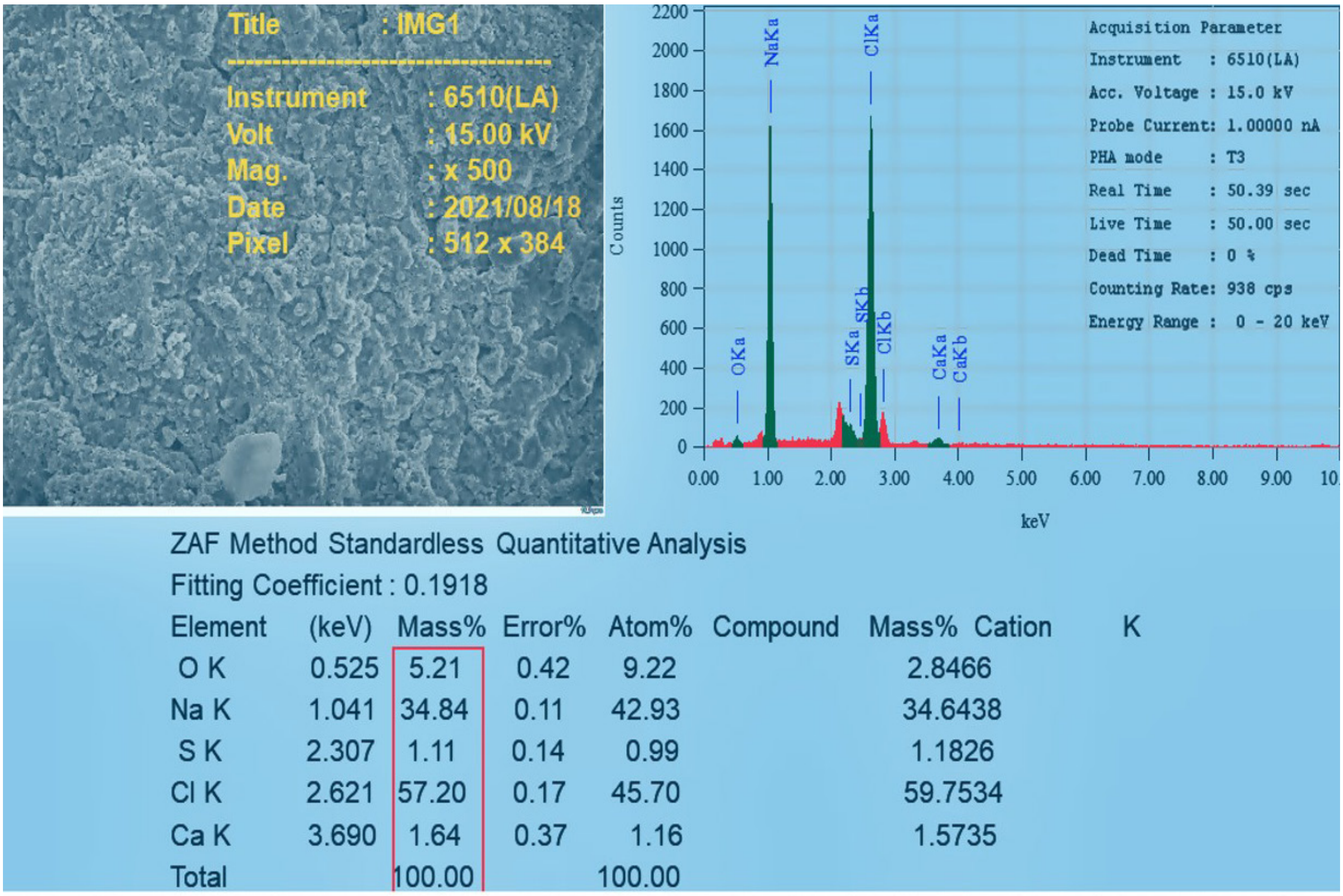
samples of red algae flour analyzed for elements showed different results, both among treatments and across elements.

Based on the same analysis, the biomineral elements of red algae flakes varied between treatments. The diversity of elements was statistically analyzed for O, Na, Cl, and Mg, as presented in Table 2 and Figure 4. Table 2 explains that concentrations of organochlorine have no significant effect on the element content of red algae ($p > 0.05$), thus indicating no interaction between elements O, Na, Mg, and S. The element content at each treatment seemed to be following the percentage value of the EDS analysis (Figure 5).

Table 2. ANOVA on the effect of organochlorine on the element content of red algae.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	9774.377[a]	19	514.441	5.135	.000	.710
Intercept	15.530.712	1	15.530.712	155.553	.000	.795
Doses	230.937	4	57.734	.578	.680	.055
Element	7.911.264	3	2.637.088	26.413	.000	.665
Doses * Element	1.632.176	12	136.015	1.362	.224	.290
Error	3.993.681	40	99.842			
Total	29.298.770	60				
Corrected Total	13.768.058	59				

Note : a. R Squared = .710 (Adjusted R Squared = .572)



Element	(keV)	Mass%	Error%	Atom%	Compound	Mass%	Cation	K
O K	0.525	5.21	0.42	9.22				2.8466
Na K	1.041	34.84	0.11	42.93				34.6438
S K	2.307	1.11	0.14	0.99				1.1826
Cl K	2.621	57.20	0.17	45.70				59.7534
Ca K	3.690	1.64	0.37	1.16				1.5735
Total		100.00		100.00				

Figure 4. Output of EDS analysis for red algae.

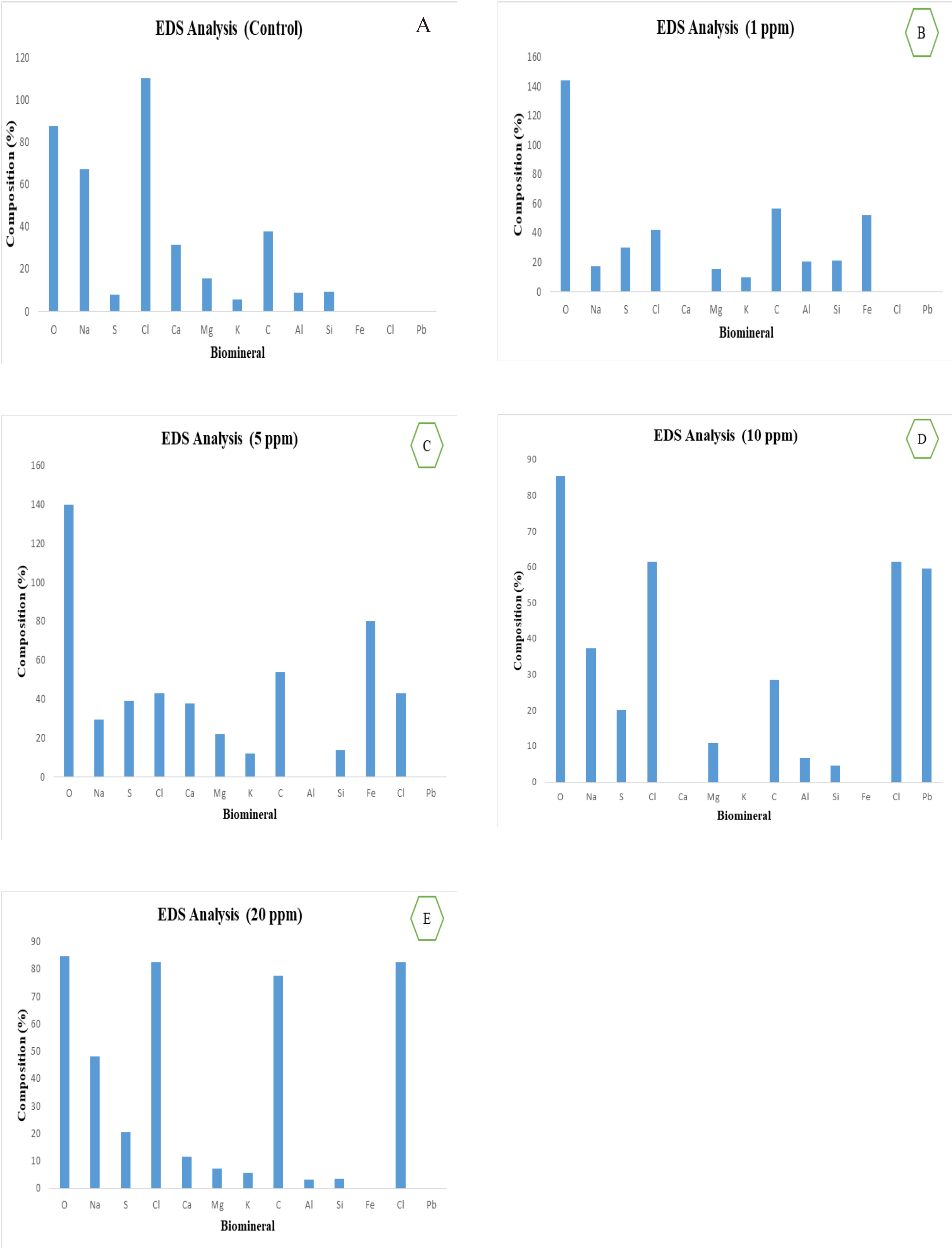


Figure 5. Mean biomineral content of *E. denticulatum* based on EDS analysis.

3.2 Discussion

3.2.1 Composition of biomineral elements

The results of the EDS analysis showed that the control was dominated by Cl (chlorine) at 34.83%, followed by O (oxygen) at 23.29%, Na (sodium) at 21.65%, and the lowest content was Si (silicon) at 0.10%. At 1 ppm, the most extensive biomineral content was the element O at 42.71%, C (carbon) at 15.05%, Cl at 13.20%, and the lowest was Fe (iron) at 0.36%. At 5 ppm, the most extensive biomineral content was the element O at 38.1%, C at 25.3%, Chlorine at 12.57%, and the lowest Fe at 0.63%. At 10 ppm, the most extensive biomineral content was element O at 25.78%, C at 21.11%, Cl at 19.57%, and the lowest Ca (calcium) at 0.09%, and 20 ppm, the most extensive biomineral content was element O at 26.63%, Cl at 25.67%, and C at 25.05%, while the lowest was Ca at 0.36%.

3.2.2 Comparative findings

It is reported that the green algae *Halimeda macroloba* contains elements of biomineral com pounds dominated by more than 40% C (carbon) (Kepel *et al*., 2021). In addition to the element carbon, *H. macroloba* also contains 55.19% O (oxygen), 6.03% Na (sodium), 32.16% Ca (calcium), and 6.62% Cl (chlorine). This is due to the exchange of ions in the body of algae. Green algae *H. opuntia* contains biomineral elements dominated by more than 40% C (carbon). In addition to the element carbon, *H. opuntia* is also composed of 47.29% O (oxygen), 30.30% Ca (calcium), and 22.41% C (carbon).

Another report by (Singkoh *et al.,* 2019) explained that the red algae *Tricleocarpa fragilis* contained more than 40% of biomineral compound elements dominat ed by C (carbon). Of the 6 specimens analyzed using EDS, the average biomineral compounds present in *A. fragilis*, in addition to the carbon element, are 39.86% O (oxygen), 14.5% Ca (calcium), Pb (lead) 3.53%, Pt (platinum) 3.13%, S (sulfur) 1.5%, Ni (nickel) 0.34%, K (potassium) 0.19%, Fe (iron) 0.19%, Co (cobalt) 0.19%, Zn (zinc) 0.16%, Mg (magnesium) 0.15%, Na (sodium) 0.14%, Al (aluminum) 0.12%, Mn (manganese) 0.09%, Cr (chromium) 0.05%, Se (selenium) 0.04%, and P (phosphorus) 0.02%.

4. Conclusion

Organochlorine influences the nanoparticle and the thickness of red algae cellulose. SEM analysis with EDS visualization showed an accumulation of Chlorine (Cl) in the thallus of red alga. This research is essential to prevent and reduce the disposal of organochlorines into the environment because they can accumulate in soil, water, and air and can also remain for years in the environment, which in turn will accumulate in food chains and negatively affect ecosystems and marine animals. Future research must examine the correlation between organochlorine and biomineral content and establish the safe organochlorine concentration for red algae.

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Authors' Contributions

The author's contribution to this research is as providing private funding, sample collection, data analyst, article writer and translation.

Conflict of Interest

The authors declare that they have no conflicts of interest regarding the publication of this article. This research was conducted in compliance with the ethical standards of Sam Ratulangi University.

Declaration of Artificial Intelligence (AI)

The author(s) affirm that no artificial intelligence (AI) tools, services, or technologies were employed in the creation, editing, or refinement of this manuscript. All content presented is the result of the independent intellectual efforts of the author(s), ensuring originality and integrity.

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