

Morphology and chemical elements contained in *Dunaliella salina* cells exposed to lead acetate using Scanning Electron Microscopy (SEM)

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Abstract. This study aimed to characterize changes in surface morphology of the marine microalga *Dunaliella salina* following lead acetate exposure using Scanning Electron Microscopy (SEM) and to determine its elemental composition using Energy Dispersive Spectroscopy (EDS). The work is intended to improve understanding of *D. salina* responses to lead contamination and to support environmental monitoring and management of heavy-metal pollution. The *D. salina* culture was obtained from a laboratory stock maintained at the Laboratory of Molecular Biology and Marine Pharmaceuticals, FPIK UNSRAT. Cells were motile and displayed morphological variability, ranging from elliptical to cylindrical forms, with radially symmetric cells measuring approximately 2.8-40 μm . Seawater for the culture medium was collected from an unpolluted coastal area, filtered through a 0.45 μm membrane using an aspirator and diluted with distilled water to a salinity of 20 ppt. Cultivation was conducted in Walne medium at 25°C and 20 ppt under continuous illumination from a 20 Watt fluorescent lamp. Cultures were harvested during the exponential growth phase, which was monitored daily by hemocytometer counts under a light microscope and then exposed to lead acetate at 30, 50 and 80 ppm respectively. Harvested biomass was centrifuged prior to SEM analysis, while cell density was quantified microscopically using a hemocytometer. The results showed no significant differences in cell density among the tested lead acetate concentrations and cell density was not associated with the observed structural characteristics across treatments. However, SEM observations demonstrated that lead acetate exposure caused evident cell-wall damage and measurable alterations in the surface morphology of *D. salina*. The mineral content found in *D. salina* consisted of carbon (C), oxygen (O), sodium (Na), magnesium (Mg), silicon (Si), sulfur (S), chlorine (Cl), potassium (K) and lead (Pb).

Keywords: aquatic environment, heavy metals, microalgae, pollutants, SEM.

Introduction. Environmental pollution is one of the most critical forms of environmental degradation caused by human activities as well as natural processes. In addition to soil and air pollution, marine pollution has become a major issue faced by many countries around the world. The accumulation of heavy metals such as Hg, Cu and Pb in marine biota, including algae, can cause severe health problems in humans who consume contaminated organisms at certain concentrations, leading to neurological disorders, kidney damage and even death (Mantiri et al., 2018, 2019).

Lead acetate is one of the most prevalent heavy metal contaminants found in the environment and can enter marine ecosystems through various sources. Pollution sources commonly originate from industrial waste, mining activities, the use of chemical materials containing lead and other anthropogenic activities. Several studies have reported the harmful effects of lead on human health (Indirawati, 2017; Collin et al., 2022; Mandal et al., 2022; Maria et al., 2025). Research conducted by Mantiri et al., (2019) in North Sulawesi also revealed that lead concentrations in coastal waters were relatively high. Once released into the marine environment, this heavy metal tends to settle on the seabed or dissolve in seawater, subsequently poisoning the marine organisms inhabiting these ecosystems.

In polluted waters, microalgae play an important role as bioremediators (Fitri et al., 2021; Kadang et al., 2024). Their broad range of ecological functions and industrial applications has made microalgae the focus of extensive research in various countries. *Dunaliella salina*, a marine microalga belonging to the class Chlorophyceae, is highly responsive to osmotic changes and is known as a halotolerant microalga capable of surviving and growing under extreme environmental conditions (Oren, 2014; Kemer et al., 2020). Therefore, it is essential to conduct an in-depth investigation of the surface morphology of *D. salina* cells exposed to lead acetate using Scanning Electron Microscopy (SEM) and Energy Dispersive Spectroscopy (EDS) and to determine the chemical elements contained in the cells.

Material and Method

Test organism. The microalga *D. salina* used in this study was obtained from stock cultures maintained at the Aquaculture Technology Laboratory, Faculty of Fisheries and Marine Sciences, Unsrat. *D. salina* cells are motile and exhibit variable shapes, ranging from elliptical to cylindrical. Cell length varies depending on the morphological form of *Dunaliella*, with radially symmetrical cells measuring approximately 2.8-40 µm. The size of the cells is influenced by culture density and light intensity.

Test materials. Seawater used as the culture medium was collected from clean coastal waters far from residential areas. The seawater was filtered using a 0.45 µm Millipore filter with the aid of an aspirator and then diluted with distilled water until a salinity of 20 ppt was achieved. The culture medium was enriched by adding 1 mL of Walne medium to 1,000 mL of filtered seawater.

Experimental procedures. The *D. salina* microalgae were cultured in Walne medium inside a controlled culture room at 25°C with a salinity of 20 ppt and continuous illumination from a 20-watt fluorescent tube lamp. Harvesting was performed when the culture reached the exponential growth phase, determined by daily cell density measurements using a hemocytometer under a light microscope. Once the culture reached its exponential phase, samples were treated with lead acetate at concentrations of 0 ppm (control), 30 ppm, 50 ppm and 80 ppm respectively, each with five replications. These concentrations were selected based on preliminary tests showing that *D. salina* remained viable under stress conditions when exposed to concentrations below 30 ppm. After harvesting, the samples were centrifuged and prepared for analysis using SEM and EDS.

Data collection. The research was conducted from May to November 2025. Observations of *D. salina* cell density prior to treatment are shown in Table 1. Lead acetate treatment was applied when the microalgal culture reached the exponential phase, which occurred on day 9. The cell density before exposure to lead acetate was 18×10^4 cells mL⁻¹.

Table 1

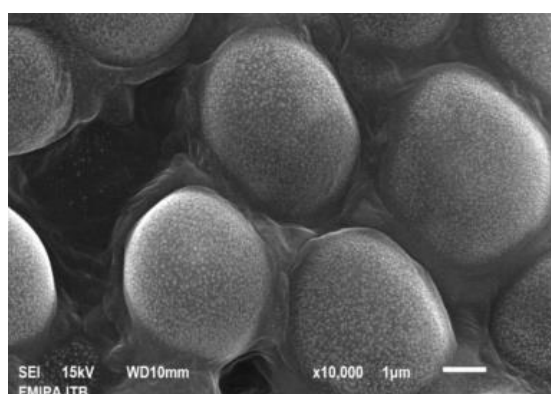
Cell density of *Dunaliella salina* before treatment

Day	Population density before treatment ($\times 10^4$ cell mL ⁻¹)					Mean
	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	
1	2	2	3	2	1	2
2	3	3	4	3	2	3
3	9	7	5	4	6	6.2
4	5	7	7	6	9	6.8
5	10	11	12	12	14	11.8
6	11	11	13	14	14	12.6
7	18	16	13	13	17	15.4
8	17	18	15	16	18	16.8
9	15	17	19	19	20	18

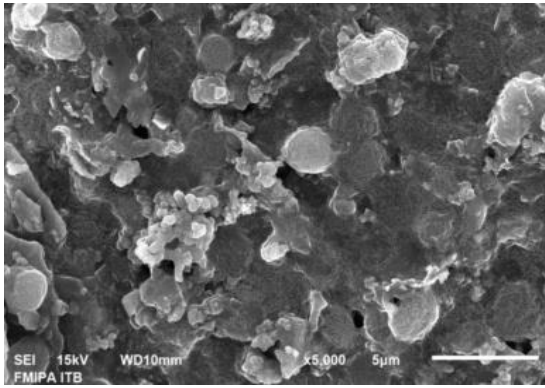
Data analysis. The morphology and chemical elements of cells were determined using SEM (JEOL JSM 6510LA) while the effect of different lead acetate concentrations on *D. salina* cells was analyzed using SPSS 24 for Windows at significant level of 0.05.

Results and Discussion. Statistical analysis showed that there were no significant differences among the treatments with different lead acetate concentrations. The cell density of *D. salina* did not exhibit any clear relationship with the cell structure observed at each concentration. Lead acetate is a highly toxic heavy metal for *D. salina* and has the potential to inhibit cell density. Increasing concentrations of lead acetate generally result in higher levels of cell mortality (Kadang et al., 2024). A similar pattern was observed in the relationship between the maximum growth rate of *Spirulina plantesis* and varying concentrations of copper (Cu). Treatments with 1 ppm Cu supported higher cell density, whereas increasing Cu concentrations resulted in progressively lower cell densities. *S. plantesis* was able to absorb copper across all treatments, with the highest absorption observed at 3 ppm, reaching 97.886%. However, treatments at 5 ppm exhibited toxic effects, leading to decreased cell density (Budi et al., 2020). Comparable findings were also reported by Kadang et al. (2024), who showed that different concentrations of lead acetate caused a reduction in cell growth and density in the microalga *Tetraselmis chuii*. Similarly, Mailoor et al. (2023) found that the growth and cell density of *Nannochloropsis oculata* decreased when exposed to lead acetate compared to the control (untreated) cultures.

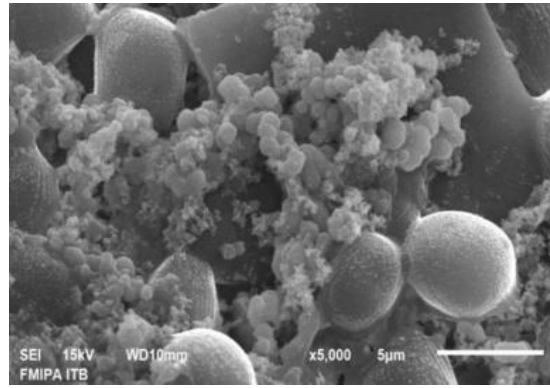
SEM/EDS analysis of *Dunaliella salina*. At the exponential phase under 30 ppm lead acetate exposure, many *D. salina* cells were still viable and maintained relatively normal structural integrity. At this concentration, visible damage was minimal. However, subsequent observations revealed mild structural disruption, particularly in the cell membrane. As shown in Figure 1, membrane damage became evident, where the membrane appeared non-intact and showed incomplete adhesion to the underlying cell structures. This deformation may indicate cytoplasmic accumulation beneath the cell membrane, likely due to the circulation and penetration of heavy metal ions into the cell, resulting in membrane swelling or blebbing. SEM analysis revealed the surface morphology of *D. salina* cells, indicating changes in cell shape and texture. The microstructural alterations observed in the SEM images suggest that lead acetate exposure induces noticeable stress responses, including membrane distortion and the formation of irregular nano-scale surface structures.



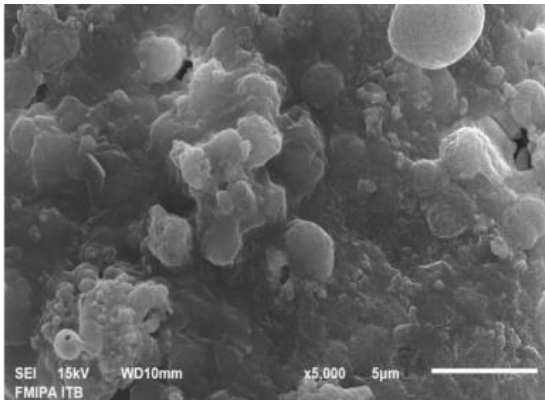
(a) Control



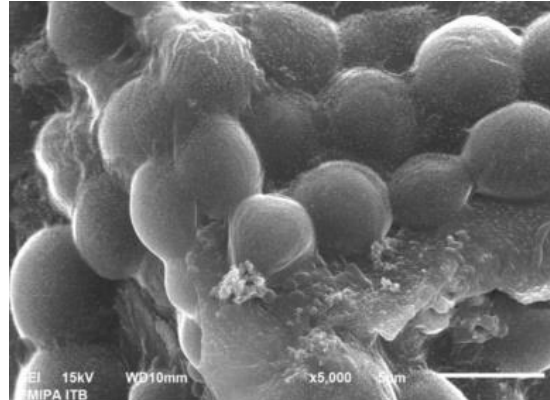
(b) Exponential phase at 30 ppm lead acetate



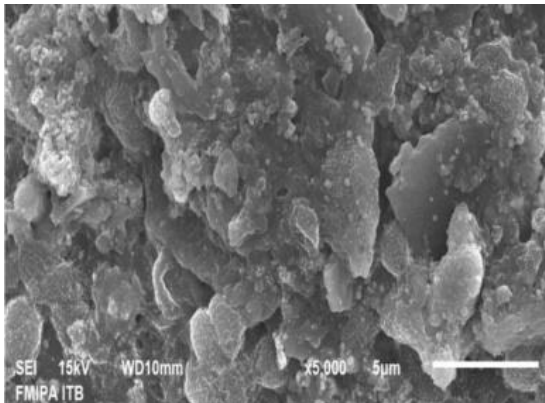
(c) Cell death phase at 30 ppm lead acetate



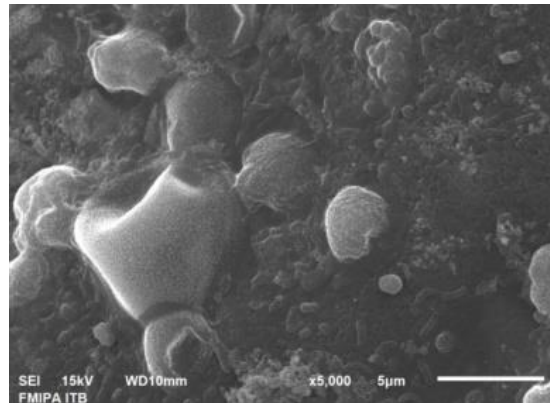
(d) Exponential phase at 50 ppm



(e) Cell death phase at 50 ppm



(f) Exponential phase at 80 ppm

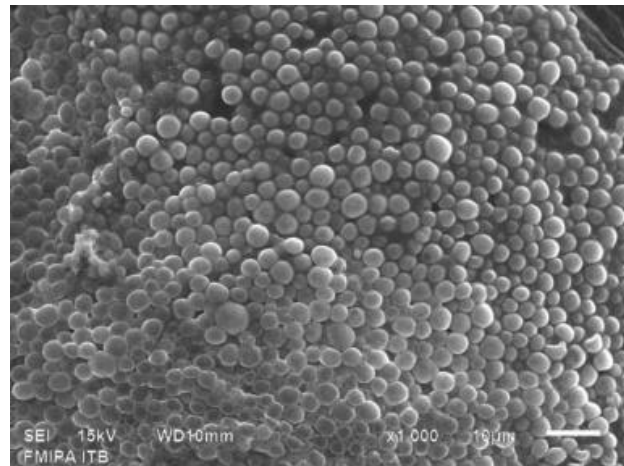


(g) Cell death phase at 80 ppm

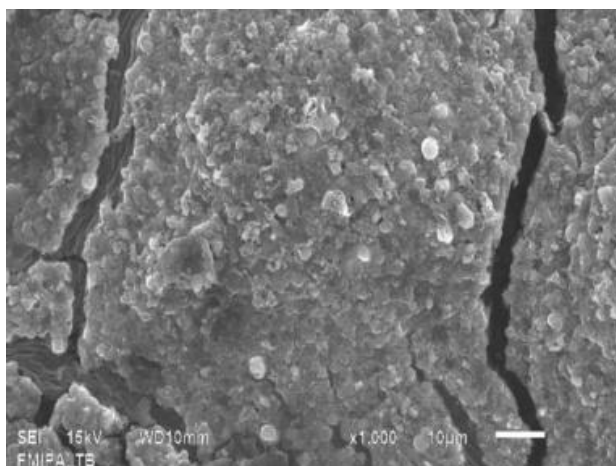
Figure 1. SEM analysis of the surface morphology of *D. salina*.

Under the control condition at 10,000 $\times$ magnification, *D. salina* cells appeared healthy and morphologically normal (Figure 2). In the exponential phase at 30 ppm, the cells showed signs of structural disorganization, with darker regions visible within the cells, indicating cellular damage at this stage. At the 30 ppm death phase (5,000 $\times$ magnification), clear distinctions were observed between cells that remained relatively intact and those that had begun to shrink or deteriorate. At 50 ppm during the exponential phase (5,000 $\times$ magnification), pronounced structural damage was evident. The affected *D. salina* cells displayed wrinkled surface textures and noticeably reduced cell size, indicating severe morphological alteration. In the 50 ppm death phase (5,000 $\times$ magnification), some cells still exhibited relatively stable structural conditions, suggesting varying levels of tolerance among individual cells. In the 80 ppm exponential phase, cell

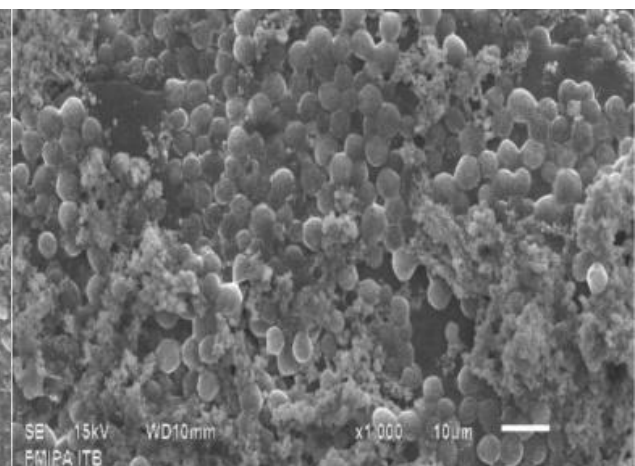
condition deteriorated further. At 5,000 $\times$ magnification, the structural and morphological damage appeared increasingly severe, with widespread deformation of the cell surface. In the 80 ppm death phase (5,000 $\times$ magnification), extensive cellular damage was clearly visible, cells showed significant shrinkage and darkened surrounding areas indicated regions of cell death around the remaining cells that managed to persist.



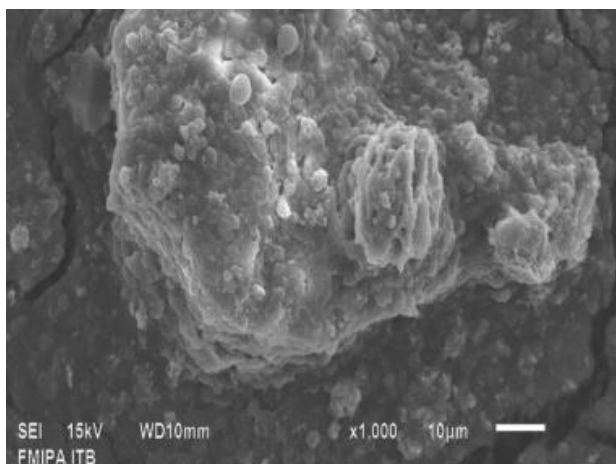
(a) EDS of the control



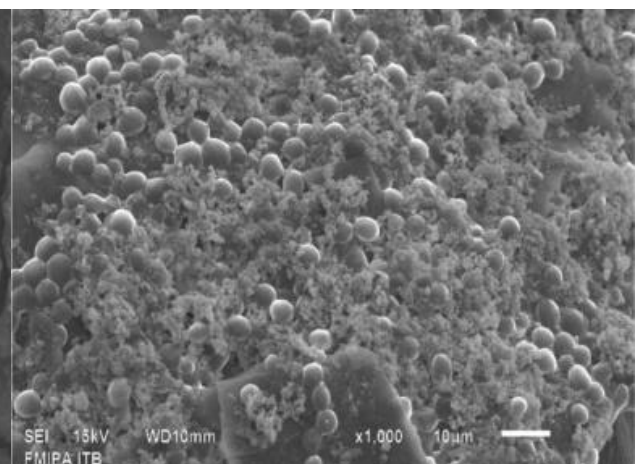
(b) EDS during the exponential phase at 30 ppm



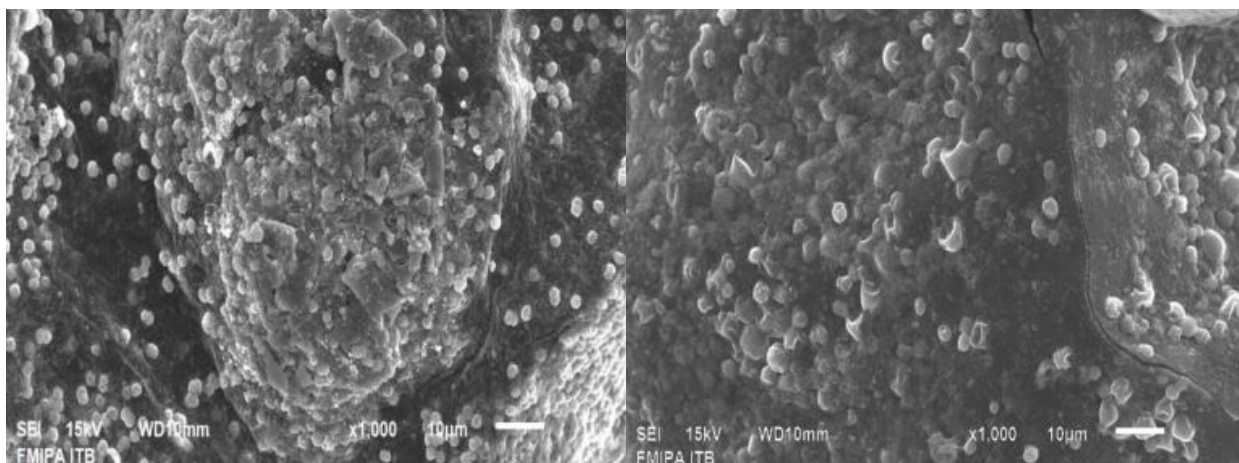
(c) EDS during the cell death phase at 30 ppm



(d) EDS during the exponential phase at 50 ppm



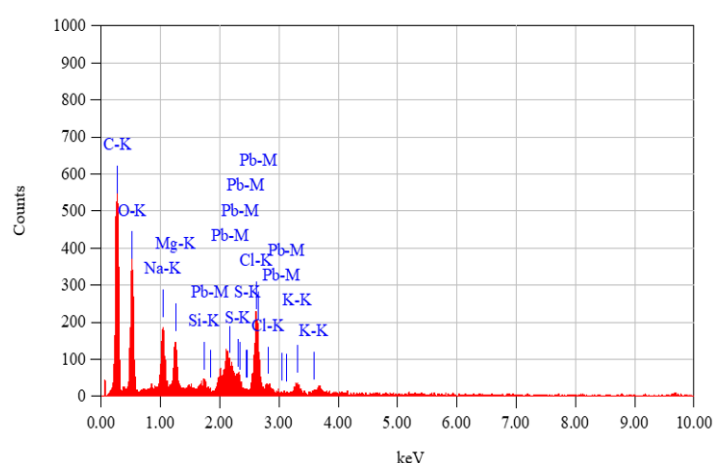
(e) EDS during the cell death phase at 50 ppm



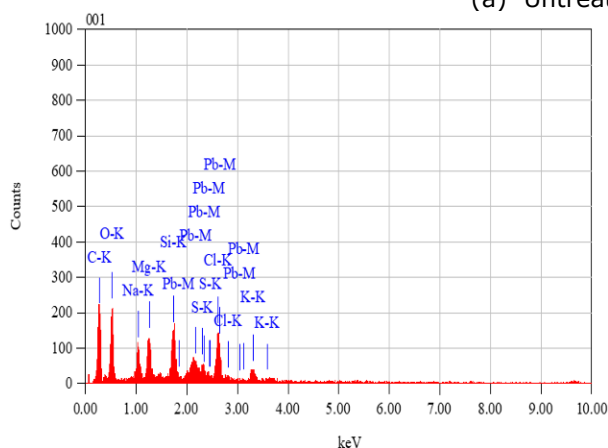
(f) EDS during the exponential phase at 80 ppm (g) EDS during the cell death phase at 80 ppm

Figure 2. EDS analysis of *Dunaliella salina* exposed to lead acetate.

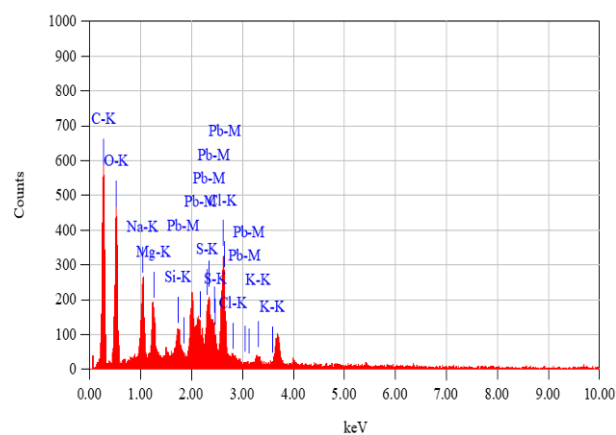
SEM chromatogram of elements contained in *D. salina* was presented in Figure 3. SEM is widely used in biological sciences and materials science to examine the surface topology and elemental composition of specimens. SEM operates by directing a focused beam of electrons that scans the specimen surface in a raster pattern. The interaction between the electron beam and the sample generates various signals, including emitted electrons and X-rays, which are processed to produce detailed surface images. These signals allow for the analysis of elemental composition and enable high-resolution imaging of biological specimens.



(a) Untreated (control)



(b) Exponential phase at 30 ppm



(c) Death phase at 30 ppm

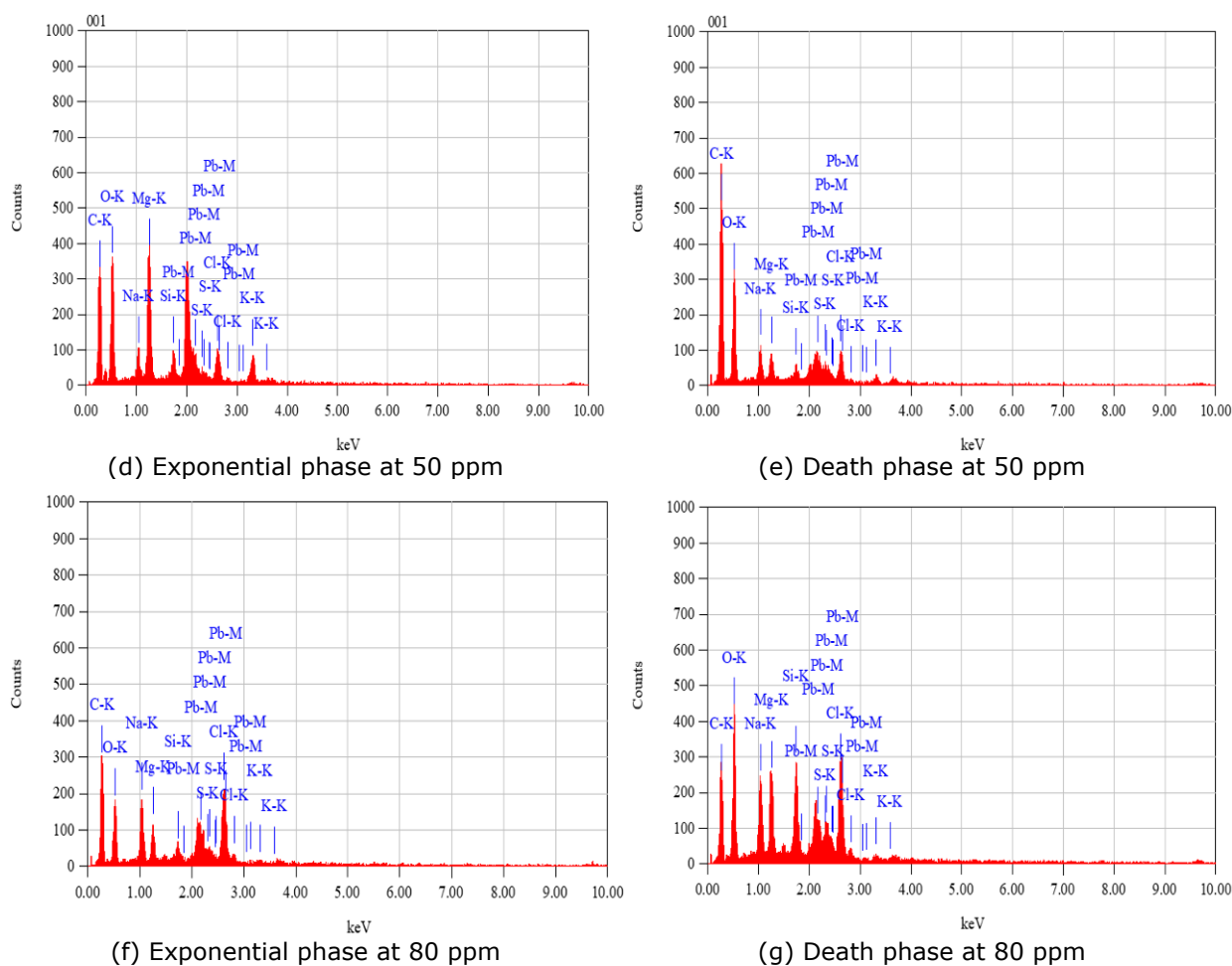
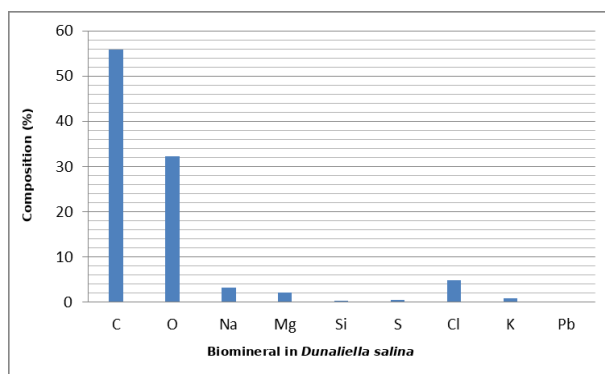
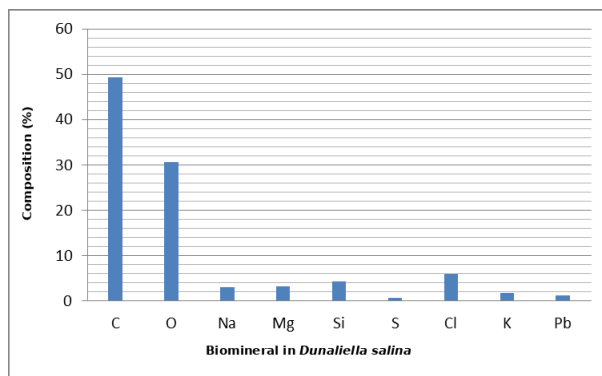


Figure 3. SEM chromatogram of elements contained in *D. salina* exposed to lead acetate.

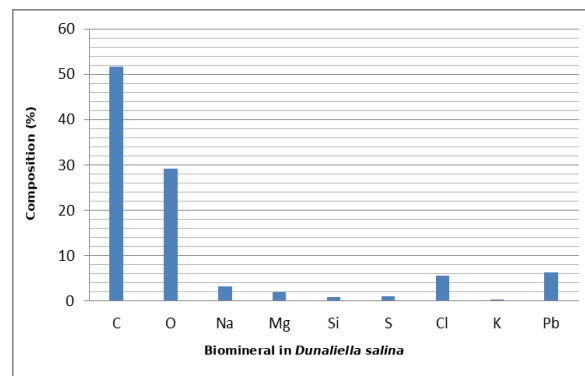
Untreated *D. salina* cells contained elements predominantly composed of carbon (C), accounting for 55.86%. In addition to carbon, *D. salina* consisted of oxygen (O) at 32.22%, sodium (Na) 3.29%, magnesium (Mg) 2.10%, silicon (Si) 0.27%, sulfur (S) 0.43%, chlorine (Cl) 4.89%, potassium (K) 0.77% and lead (Pb) at a trace level of 0.18% (Figure 4).



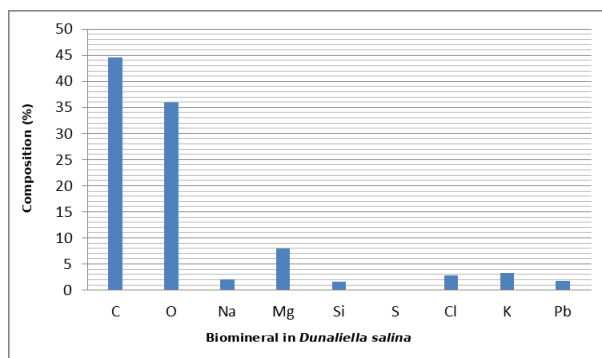
(a) Untreated control



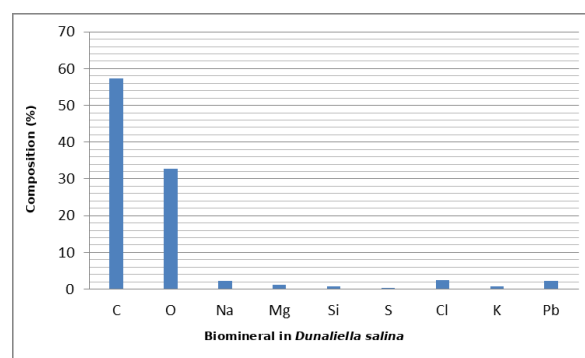
(b) Exponential phase at 30 ppm



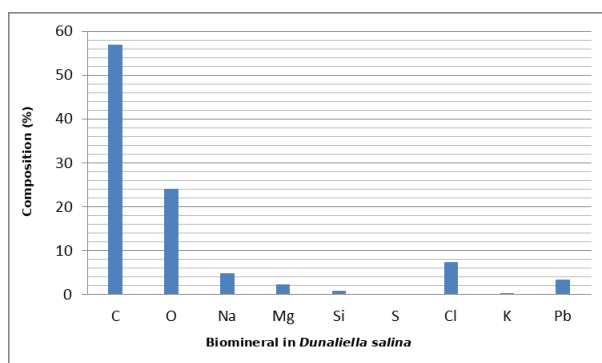
(c) Cell death phase at 30 ppm



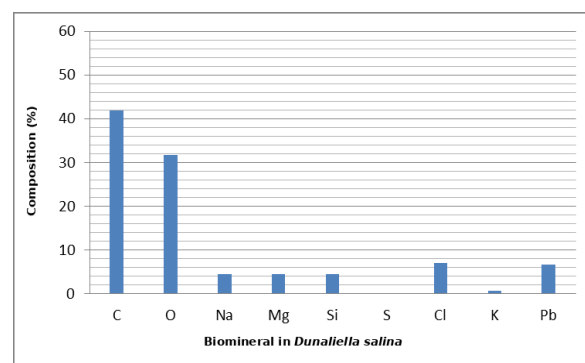
(d) Exponential phase at 50 ppm



(e) Cell death phase at 50 ppm



(f) Exponential phase at 80 ppm



(g) Cell death phase at 80 ppm

Figure 4. Elemental composition diagram from the SEM analysis of *Dunaliella salina*.

In *D. salina* treated with 30 ppm lead acetate during the exponential phase, the elemental composition was dominated by carbon at 49.23%. Other detected elements included oxygen (30.58%), sodium (3.10%), magnesium (3.20%), silicon (4.20%), sulfur (0.66%), chlorine (5.92%), potassium (1.80%) and an elevated lead concentration of 1.28%.

During the 30 ppm death phase, carbon remained dominant at 51.59%, followed by oxygen (29.24%), sodium (3.23%), magnesium (1.85%), silicon (0.81%), sulfur (0.96%), chlorine (5.64%), potassium (0.33%) and a substantially increased lead concentration of 6.34%.

At 50 ppm in the exponential phase, the elemental profile of *D. salina* showed carbon at 44.55%, oxygen at 35.88%, sodium at 2.07%, magnesium at 7.99%, silicon at 1.55%, sulfur at 0.12%, chlorine at 2.81%, potassium at 3.30% and lead at 1.74%.

During the 50 ppm death phase, carbon content increased to 57.35%, followed by oxygen (32.72%), sodium (2.15%), magnesium (1.28%), silicon (0.72%), sulfur (0.30%), chlorine (2.46%), potassium (0.85%) and lead at 2.16%.

For microalgae exposed to 80 ppm during the exponential phase, carbon was recorded at 56.99%, oxygen at 24.09%, sodium at 4.77%, magnesium at 2.35%, silicon at 0.77%, chlorine at 7.38%, potassium at 0.23% and lead at 3.41%.

In the 80 ppm death phase, carbon content declined to 41.86%, while oxygen reached 31.65%, sodium 4.15%, magnesium 4.16%, silicon 4.14%, sulfur 0.30%, chlorine 6.82%, potassium 0.48% and lead concentration increased markedly to 6.44%.

Recent reports by Gao et al. (2026) displayed mineral contents in several microalgal samples. Ca content in *D. salina* was 10,359.6-24,146.3 mg kg⁻¹, in *Tetraselmis chuii* - 12,920.8 mg kg⁻¹, in *Arthrospira platensis* - 12,920.8 mg kg⁻¹, in *Chlorella vulgaris* green - (*C. vulgaris*-G) 1651.7 mg kg⁻¹, in *Haematococcus pluvialis*-L - 1537.3 mg kg⁻¹, in *Haematococcus pluvialis*-U - 516.9 mg kg⁻¹. Mg content in *D. salina* was 15,245.9 mg kg⁻¹, in *H. pluvialis*-U - 423.0 mg kg⁻¹, in *H. pluvialis*-L - 379.2 mg kg⁻¹, in *Chlorella vulgaris* white (*C. vulgaris*-W) - 1427.8 mg kg⁻¹, in *C. vulgaris*-G - 1264.5 mg kg⁻¹, in *Chlorella vulgaris* yellow (*C. vulgaris*-Y) - 1228.2 mg kg⁻¹, *Nannochloropsis oceanica* - 7198.1 mg kg⁻¹, and in *T. chuii* - 9436.7 mg kg⁻¹. K content in *C. vulgaris*-G was 61,235.9 mg kg⁻¹ and in *H. pluvialis*-U was 9042.1 mg kg⁻¹. P content in *D. salina* was 942.6 mg kg⁻¹, while in *C. vulgaris*-W was 13,917.9 mg kg⁻¹.

The release of pollutant such as lead (Pb) from industries, agriculture wastes, mining activities and domestic wastes into the environment has threatened the marine ecosystem. This research found that Pb contained in *D. salina* increased as the concentration of Pb treatments increased. This phenomenon indicated the ability of this microalga to accumulate Pb and thus lowering the concentration of Pb in the environment. Therefore, *D. salina* could be used as a good bioindicator for Pb pollution. Microalgae *C. vulgaris* had also been reported to have the ability to absorb Pb from the environment, thus serves as a bioindicator for Pb pollution (Hee et al., 2021).

Conclusions. The cells and surface morphology of the microalga *Dunaliella salina* exposed to lead acetate showed clear signs of cell wall damage and alterations in surface structural characteristics. This study has potential to be developed as bioindicator of heavy metal pollution, especially lead acetate. It can also be used in bioremediation in polluted water environment.

Acknowledgements. The authors would like to thank all those who have contributed in this work from the beginning until completion.

Authors Contributions. KK and NK: Data collection and analysis, conceptualization; DMHM: Methodology, Review & Editing; KK: preparation original draft; DSJP: Review & Editing.

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

Data Availability. The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Funding. This research was funded by the Sam Ratulangi University through the Institute for Research and Community Service.

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Received: 29 May 2026. Accepted: 31 July 2026. Published online: 26 September 2026.

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How to cite this article:

Kemer, K., Mantiri, D. M. H., Paransa, D. S. J., & Kadang, N. (2026). Morphology and chemical elements contained in *Dunaliella salina* cells exposed to lead acetate using Scanning Electron Microscopy (SEM). *Aquaculture, Aquarium, Conservation & Legislation*, 19(5), 2226-2236.