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MEASUREMENT AND RESEARCH METHODOLOGY GUIDE

Seawater, Sediment and Macroalgae Sampling: Advanced Applications in Fertilizer and Bioplastic Production

SİNOP ÜNİVERSİTESİ 2007

MARMARA MUNICIPALITIES UNION

КОНСТАНТИН ПРЕСЛАВСКИ ШУМЕНСКИ УНИВЕРСИТЕТ SHUMEN UNIVERSITY



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Seawater, Sediment, and Macroalgae Sampling: Advanced Applications in Fertilizer and Bioplastic Production

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Activity 1.1. & A.1.2
Establishing Research Team and Research Methodology I & II

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EXECUTIVE SUMMARY

The MACRO CLEAN (*Research, Cleaning Methodologies, Economic Valorization and Pilot Application for Macroalgal Blooms*) Project, implemented under the Interreg NEXT Black Sea Basin Programme (BSB00584), is a collaborative initiative involving Sinop University (SNU) and Marmara Municipalities Union (MMU) from Türkiye, Batumi State University (BSU) from Georgia, and Shumen University (ShU) from Bulgaria. The project focuses on the economic utilization of macroalgae through research and pilot implementations, while also aiming to prevent and mitigate the impacts of human activities contributing to eutrophication and macroalgae blooms in the Black Sea region.

The main objective of the project is to develop a comprehensive research methodology for macroalgae studies, enhance laboratory capacities, prepare feasibility plans for macroalgae-based applications, raise awareness among stakeholders, and foster collaboration on innovative cleaning technologies. By addressing the regional challenges of eutrophication and algal blooms — largely caused by land-based pollution and changes in marine ecosystems — the project promotes sustainable resource management and seeks to create lasting positive impacts.

This guide outlines the methodology collaboratively developed by Activity 1.1 and Activity 1.2 for sampling macroalgae, seawater, and sediments, as well as for utilizing macroalgae in fertilizer and bioplastic applications. Through stakeholder engagement and the development of a harmonized methodology, A.1.1. and A.1.2 has produced a set of practical guidelines designed to assist policymakers, researchers, and environmental organizations in monitoring and implementing these activities effectively.

While this document reflects the collective efforts and informal consensus of all contributing partners, it does not necessarily represent the official views of the Interreg NEXT Programme.

Prof. Dr. Levent BAT
Project Coordinator
September, 2025

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INTRODUCTION

Rationale

The MACRO CLEAN Project was initiated to address pressing environmental challenges through the collaborative efforts of partners from three Black Sea Basin countries: Türkiye, Georgia, and Bulgaria. This project establishes a comprehensive framework for research and pilot applications focused on the sustainable and effective collection, evaluation, and economic valorization of macroalgae, targeting their use as fertilizers and bioplastics.

To this end, a jointly developed methodology provides clear guidelines for sampling macroalgae, seawater, and sediments, as well as for their utilization in various economic sectors. This guide presents an integrated research methodology encompassing four main components: field studies, sampling techniques, analytical procedures, and applied research.

Moreover, it highlights practical applications and innovative approaches to macroalgae utilization, illustrated through case studies and pilot projects related to fertilizers and bioplastics. By ensuring methodological consistency and enhancing data quality, these components aim to facilitate knowledge sharing among researchers, institutions, and stakeholders committed to promoting the sustainable use of marine resources within the Black Sea region and beyond.

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ACRONYMS

AAS	Atomic Absorption Spectroscopy
AFM	Atomic Force Microscopy
Al	Aluminum
Ca	Calcium
$CaCl_2$	Calcium Chloride
Co	Cobalt
Cr	Chromium
Cu	Copper
DO	Dissolved Oxygen
E223	Sodium Metabisulfite
EC	Electrical Conductivity
EVA	Ethylene-Vinyl Acetate
Fe	Iron
FTIR	Fourier-Transform Infrared
HCl	Hydrochloric Acid
Hg	Mercury
HNO_3	Nitric Acid
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
IR	Infrared
K	Potassium
KOH	Potassium Hydroxide
Mg	Magnesium
Mn	Manganese
N	Nitrogen
Na	Sodium
Na_2CO_3	Sodium Carbonate
Ni	Nickel
P	Phosphorus
ppm	Parts per million
Sb	Antimony
Se	Selenium
SEM	Scanning Electron Microscope
Sn	Tin
TGA	Thermogravimetric Analysis
UV	Ultraviolet
W/V	Weight/Volume
XRD	X-ray Diffraction
Zn	Zinc

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PART I – FIELD STUDIES

I.I. MACROALGAE BIOMASS SURVEY AND SAMPLING TECHNIQUES

A macroalgae biomass survey is conducted at coastal water points, with samples taken from at least three different locations within an area of approximately 15 x 15 meters and at a depth of 0–1 meter, to ensure regional representativeness (Figures 1 and 2).

To collect the samples, a 20 x 20 cm quadrat (400 cm^2) is used, as described in the methodology by Bouiadjra et al. (2021) (Figure 3a). Samples are manually collected using a spatula, initially placed in plastic bags, and subsequently transferred to plastic jars filled with seawater (Figure 3b). For preservation, a formaldehyde–seawater solution is added at a concentration of 2–5%, as recommended by ÇŞB-ÇEDİDGM and TÜBİTAK-MAM (2017) (Figure 3c).



Figure 1. Biomass survey

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Figure 2. Macroalgae sampling

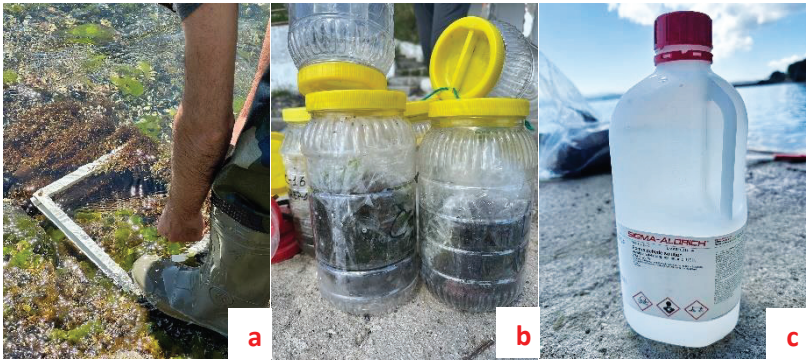


Figure 3. (a) Quadrate, (b) Plastic jars and (c) Formaldehyde

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Once in the laboratory, the samples are rinsed with distilled water to remove any foreign materials, such as stones, sand, or epiphytes (Figure 4). The cleaned samples are placed on blotting paper to remove excess water (Figure 5) and then dried in an oven at 60 °C for 48 hours (Srijaya et al., 2010; Fernández-Aláez & Fernández-Aláez, 2011; Setthamongkol et al., 2015) (Figure 6). After drying, the macroalgae are weighed, and taxonomic identification is performed on specimens that have been separated from foreign materials, using microscopic examination when necessary.

For macroalgae intended for chemical analysis, separate samples are collected independently of the biomass survey. These samples are rinsed with deionized water, cleaned of macroscopic epizoa and epiphytes, and then dried in a laboratory oven at 60 °C for 48 hours. The dried material is subsequently ground into a fine powder using a mortar and pestle (Figure 7). This process ensures that the macroalgae samples are accurately prepared for both biomass quantification and detailed chemical analysis.

Biomass measurements of the identified algal samples are carried out using an analytical balance with a sensitivity of 0.0001 g. Quantitative samples of macroalgae are converted into biomass per unit area (g dry weight/m^2). To better understand the seasonal distribution patterns of benthic algal communities along the coastline, the mean values of the macroalgal species data are subjected to statistical analysis. Additionally, in situ measurements of environmental parameters such as pH, temperature, salinity, and dissolved oxygen will be conducted using a multiparameter device (Figure 8).

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Figure 4. Cleaning and species identification



Figure 5. Removal of excess water from samples on paper



Figure 6. Drying the samples in an oven



Figure 7. Powdered samples

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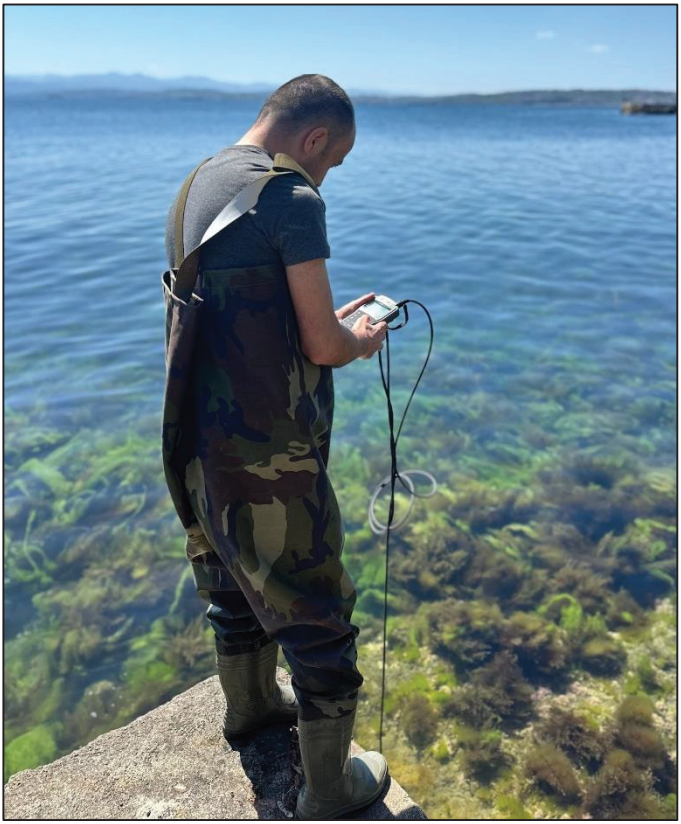


Figure 8. *In-situ measurement of physical parameters*

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I.II. SELECTION OF SAMPLING AREAS FOR MACROALGAE RESEARCH

Stations are selected based on the following criteria:

a. Coastal Characteristics: Diverse substrate types, such as sandy, rocky, and muddy environments, are targeted for sampling. These varied substrates play a significant role in the distribution and growth of macroalgae.

b. Proximity to Human Activities: Areas with high anthropogenic influence (*e.g.*, agricultural runoff, urban wastewater discharges) and less impacted, relatively pristine areas are selected. This contrast allows for the evaluation of human-induced effects. In order to understand the full scope of the environmental impact on macroalgal growth and coastal ecosystems, it is crucial to select both areas with high anthropogenic influence and those that remain relatively undisturbed. Areas affected by agricultural runoff and urban wastewater discharges often experience elevated nutrient levels, such as nitrogen and phosphorus, which contribute to eutrophication and macroalgal blooms. These areas offer valuable insights into the direct consequences of human activities on marine ecosystems. In contrast, less impacted and relatively pristine areas serve as reference sites where natural processes dominate, providing a baseline for comparison. By contrasting these two types of environments, it becomes possible to evaluate the extent of human-induced changes and to assess the effectiveness of management strategies aimed at mitigating environmental degradation. This dual approach enhances the understanding of the ecological consequences of human activities and aids in the development of more sustainable coastal management practices.

c. Accessibility and Sampling Feasibility*:* Locations are easily accessible throughout the year and suitable for repeated seasonal sampling efforts to ensure consistent monitoring and data collection.

d. Representativeness*:* The selected stations accurately reflect the general ecological status of the coastline. They are suitable for long-term monitoring, enabling continuous or repeated data collection over extended periods. This long-term approach is essential for tracking changes in the ecosystem, identifying trends over time, and evaluating the effectiveness of management or conservation efforts.

e. Spatial Variability*:* Stations are distributed at sufficient distances to capture spatial heterogeneity. This helps analyze variability across different regions and environmental gradients.

f. Resource Potential: Areas with macroalgal diversity and biomass are prioritized. Specifically, regions with species suitable for fertilizer and bioplastic production are preferred.

I.III. THE LOCATION OF THE PLANNED STATIONS

This study is designed to investigate the origins and dynamics of macroalgal overgrowth and eutrophication in the region, with a focus on identifying the environmental and anthropogenic factors that contribute to these phenomena.

A key objective of the study is to support the sustainable utilization of macroalgae through applications such as fertilizer and bioplastic production. The research specifically concentrates on the dominant macroalgal species in the region, particularly *Ulva* spp. and *Cystoseira* sp. (Figures 9 and 10). These species are sampled to assess various factors, including the density and seasonal growth patterns of macrobenthic algae, as well as the nutrient composition of macroalgae, seawater, and sediment.



Figure 9. *Ulva* sp.



Figure 10. *Cystoseira* sp.

As outlined in the study design, sampling regions were selected based on predefined criteria. In Sinop province (Türkiye), the chosen sites include Inceburun, Hamsilos, Akliman, Outer Port (Dış Liman), Karakum, Inner Port (İç Liman), and Mobil (Figure 11). In Bulgaria, the sampling locations are situated south of Cape Emine and encompass Burgas Bay, Chengene Skele Bay, Maslen Nos Bay, Petrol Harbor Bay, Sozopol and Bamboo (Figure 12). In Georgia, the designated sampling areas consist of Sarph, Kvariati, Gonio, Green Cape, Tsikhisdziri, Photi, and Kulevi (Figure 13). The coordinates (latitude and longitude) of the sampling stations are presented in the table

Table 1. The coordinates (latitude and longitude) of the sampling stations

Station name	Latitude	Longitude
İnceburun (TR)	42°5'46.38"N	34°56'40.63"E
Hamsilos (TR)	42°3'38.14"N	35°2'39.74"E
Akliman (TR)	42°3'3.31"N	35°2'37.19"E
Outer Port (TR)	42°1'51.48"N	35°9'6.32"E
Karakum (TR)	42°0'56.16"N	35°11'11.94"E
Inner Port (TR)	42°1'13.07"N	35°9'34.01"E
Mobil (TR)	42°0'36.81"N	35°7'9.87"E
Burgas Bay (BG)	42°30'16.24"N	27°29'2.87"E
Chengene Skele Bay (BG)	42°15'43.24"N	27°18'51.55"E
Maslen Nos Bay (BG)	42°18'27.17"N	27°47'24.32"E
Petrol Harbor Bay (BG)	42°26'45.36"N	27°32'4.85"E
Sozopol (BG)	42°25'20.02"N	27°41'40.29"E
Bamboo (BG)	42°24'35.49"N	27°42'20.62"E
Sarph (GE)	41°5'39.9"N	41°5'20.0"E
Kvariati (GE)	41°5'40.6"N	41°5'52.8"E
Gonio (GE)	41°5'43.85"N	41°5'56.95"E
Green Cape (GE)	41°6'96.68"N	41°7'06.14"E
Tsikhisdziri (GE)	41°7'61.02"N	41°7'42.99"E
Photi (GE)	42°0'43.66"N	41°7'27.34"E
Kulevi (GE)	42°2'48.23"N	41°6'27.75"E

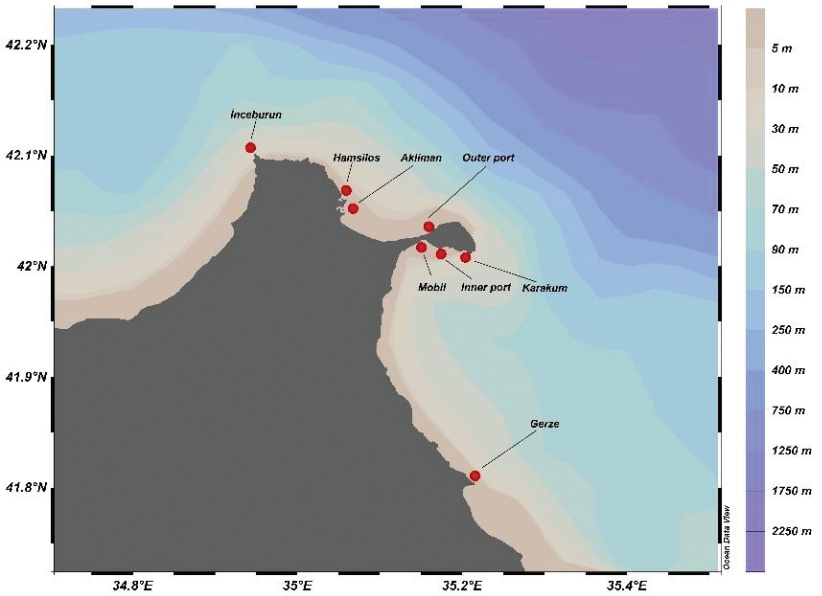


Figure 11. Sampling stations in Sinop Province

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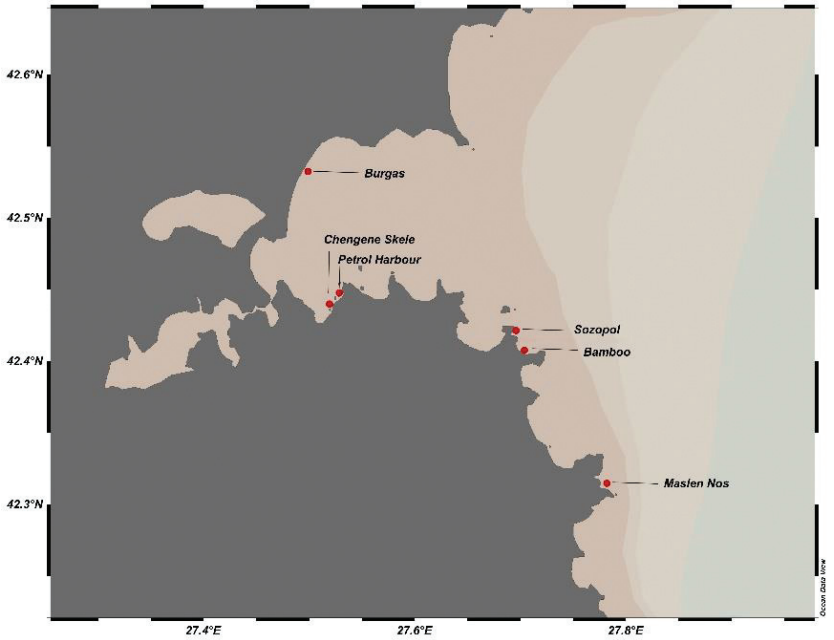


Figure 12. Sampling stations in Bulgaria

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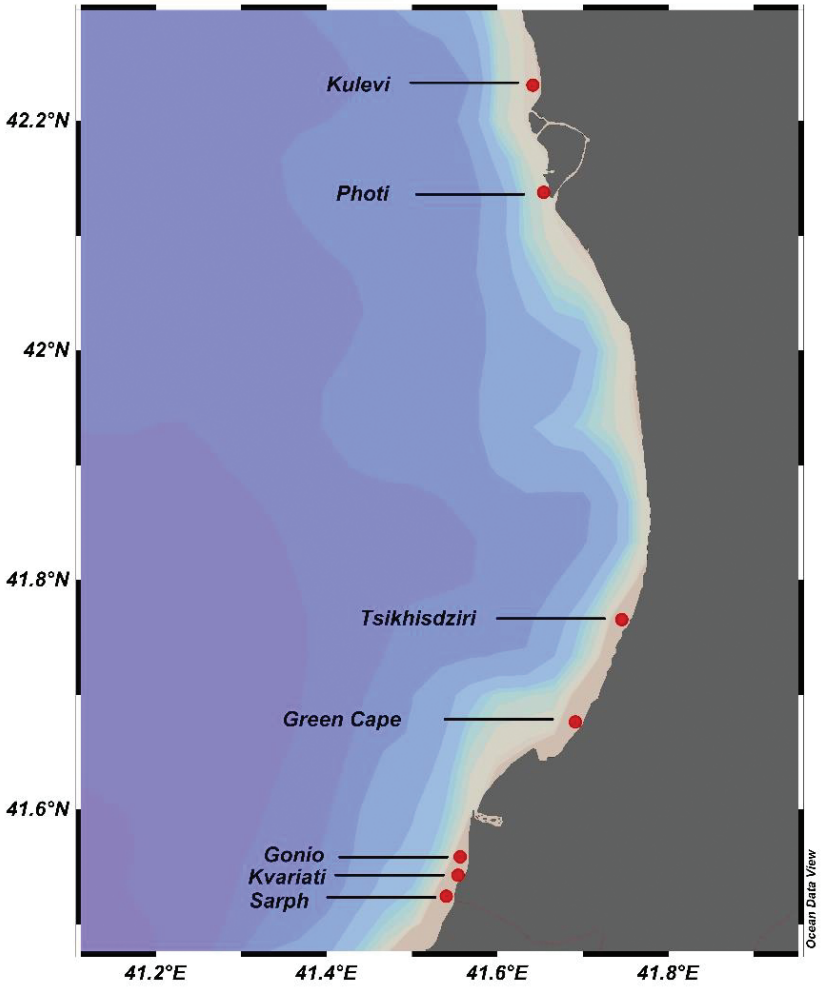


Figure 13. Sampling stations in Georgia

PART II - SAMPLING TECHNIQUES

Macroalgae, Seawater & Sediment

II.I. MACROALGAE SAMPLING

Macroalgae samples are collected seasonally from various locations along the coastline to ensure they represent the entire area being studied. The collected samples are carefully labeled and placed in polyethylene bags for transport. To preserve the samples for subsequent metal analysis, 1 ml of concentrated nitric acid (HNO_3) is added to each sample to prevent metal precipitation and maintain the solubility of trace elements. The samples are then stored at -20°C until they are ready for analysis. This method ensures the integrity of the samples, safeguarding them from degradation and contamination before they are processed in the laboratory for metal analysis (Figure 14).

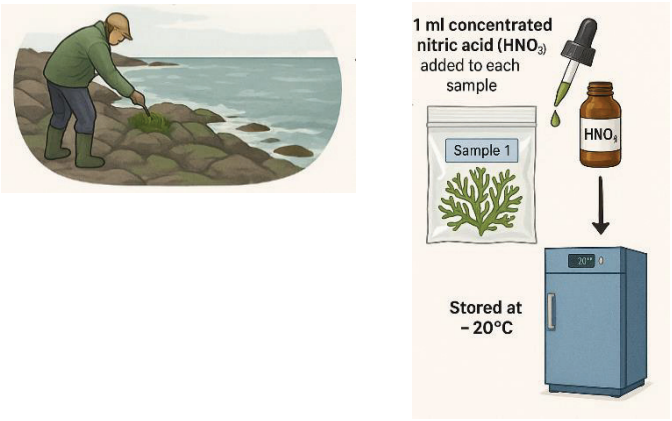


Figure 14. Illustration of macroalgae sampling

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II.II. SEAWATER SAMPLING

Seawater samples are collected from locations where macroalgae are present. These locations are selected to better understand the correlation of metal translocation between seawater and the macroalgae. In addition to sample collection, real-time in situ measurements of key physical parameters, such as temperature, salinity, and pH, are recorded using a multiparameter probe. This provides a comprehensive understanding of the environmental conditions at the time of sampling.

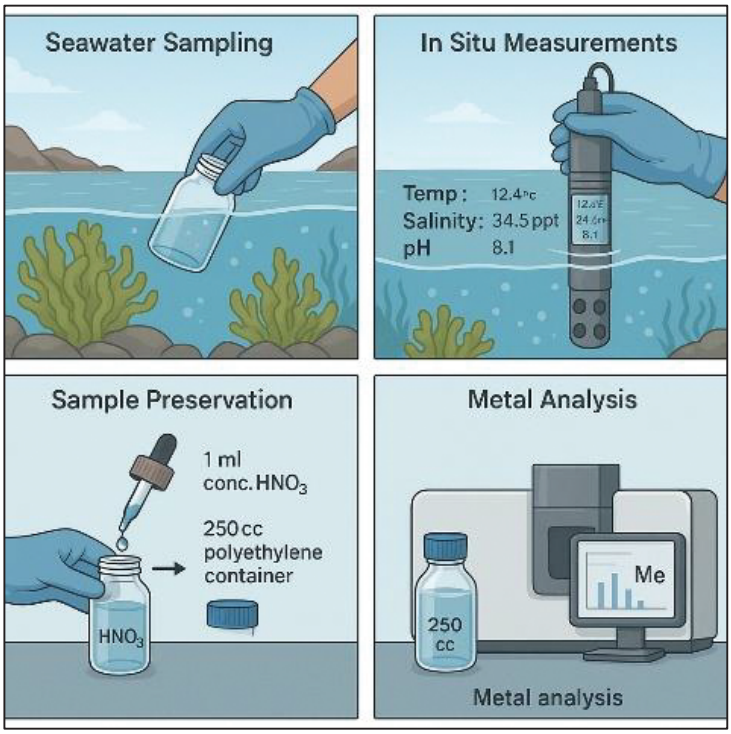


Figure 15. Illustration of seawater sampling

Seawater samples were collected in triplicate into 500 ml polyethylene containers from locations where macroalgae are present, for both metal analysis and chlorophyll-*a* (Chl-*a*) measurement.

To preserve the samples for subsequent metal analysis, 1 ml of concentrated HNO_3 is added to each container. The sealed containers are stored at room temperature, away from direct sunlight and heat sources, to maintain the stability of the samples until they are ready for laboratory analysis. The water samples used for Chl-*a* analysis were filtered using a vacuum filtration system after being brought to the laboratory (Figure 16).



Figure 16. Filtration system

Chl-a determination was carried out according to the method given below:

For each analysis, 2 liters of water samples were filtered in triplicate using GF/C glass fiber filter papers with a pore size of 0.45 µm. Prior to filtration, a few drops of 1% magnesium carbonate ($MgCO_3$) solution were added to moisten the filter papers to prevent acidification and help stabilize the pH of particles retained on the filter. The moistened filters were then placed in a vacuum filtration system, and the 2-liter samples were filtered under vacuum, allowing algae and other particulates to accumulate on the filter surface.

After filtration, the filter papers were carefully folded and placed into 15 ml centrifuge tubes (Figure 17). Each tube was then filled with 10 ml of 90% acetone solution, sealed, and stored in a refrigerator (approximately 4°C) overnight (about 12–24 hours) to facilitate the extraction of chlorophyll into the solvent. This step ensures maximum transfer of chlorophyll from algal cells into the acetone.

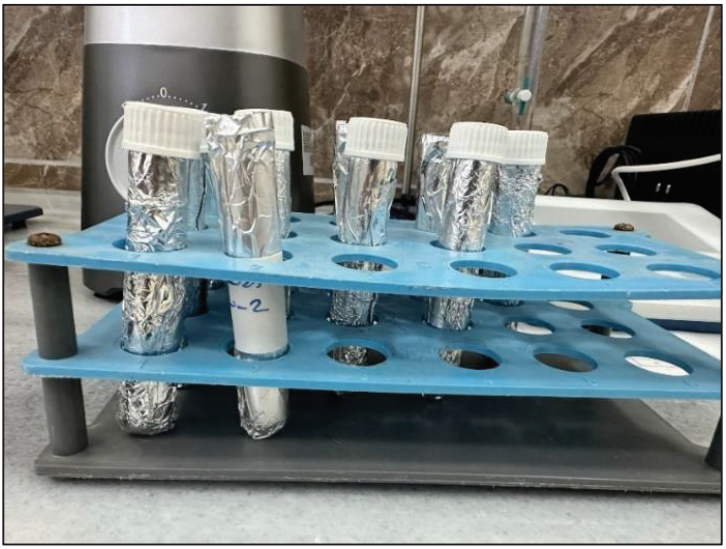


Figure 17. Centrifuge tubes

Following the 24-hour extraction period, the tubes were removed from the refrigerator and allowed to reach room temperature. They were then centrifuged at 5000 rpm for 10 minutes to sediment the solid particles and obtain a clear supernatant.

The absorbance of this clear supernatant was measured at 750, 664, 647, and 630 nm using a spectrophotometer. To eliminate errors due to turbidity (light scattering by suspended particles), the absorbance at 750 nm was subtracted from the readings at 664, 647, and 630 nm. This correction minimizes interference from non-chlorophyll light scattering, yielding more accurate chlorophyll measurements (Figure 18).

Calculation of Chl-*a* concentration:

Chl-*a* ($\mu g\ L^{-1}$) = (11,85 * (E664 – E750)-1,54 * (E647 - E750)- 0,08 * (E630-E750))*Ve/Vf

Ve: Extraction volume (ml), Vf: Volume of filtered water (liters)



Figure 18. Chlorophyll measurements

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II.III. SEDIMENT SAMPLING

Sediment samples are collected from stations with varying ground structures, using a sediment-core or shovel (Figure 19). Samples are collected from the top 0-5 cm of the sediment surface to study environmental interactions. Once collected, the samples are carefully labeled for identification and stored in a deep freezer at -20°C to preserve their integrity until further analysis (Figure 20).



Figure 19. Sediment corer

To ensure the accuracy and sensitivity of metal analyses, the sediment samples are preserved in an acidic environment. A small amount (1-2 ml) of concentrated HNO_3 is added to each sample to prevent the precipitation of metals and maintain their solubility. This step is crucial for accurate measurement of trace metals, especially when dealing with complex sediment matrices. Once treated with acid, the samples are stored at low temperatures, typically around 4°C, until they are ready for transportation to the laboratory for further processing.



Figure 20. Labeled sediment samples

Upon arrival at the laboratory, the sediment samples are dissolved using acidic solvents, such as concentrated HNO_3 or HCl, in order to break down the sediment and release the metals contained within. This process ensures that the metal content is fully extracted for analysis. The dissolved samples are then analyzed for trace metal concentrations. The analysis is conducted using either an Inductively Coupled Plasma Mass Spectrometer (ICP-MS) or Atomic Absorption Spectroscopy (AAS), both of which are highly effective techniques for detecting and quantifying trace metals in environmental samples.

PART III- ANALYTICAL TECHNIQUES

III.I. METAL ANALYSIS OF MACROALGAE

The process of metal analysis in macroalgae begins with the collection of samples from selected locations where macroalgae are abundant. Once collected, the macroalgae samples are carefully cleaned to remove seawater, debris, and any other contaminants that could interfere with the analysis. This cleaning process is critical to ensure that no external materials influence the results.

After cleaning, the macroalgae are dried in an oven. This drying process removes the moisture content. Once the algae are fully dried, they are ground into a fine powder. Typically, 0.5 to 1 gram of the dried and ground macroalgae is used for the metal analysis.

The ground seaweed is then subjected to an acidic digestion process. The sample is dissolved in concentrated HNO_3 or HCl to break down the organic material and release the metals contained within. After digestion, the solution is filtered to remove any solid residues that may remain.

The filtered solution is then diluted to the appropriate concentration and prepared for metal analysis. If the macroalgae samples need to be stored before analysis, they are kept in an acidified solution and refrigerated at 4°C to maintain their integrity until they are ready for testing.

III.II. METAL ANALYSIS OF SEAWATER

The metal analysis of seawater begins by collecting seawater samples from selected locations, particularly where macroalgae are found. Seawater samples are collected in 250 cc containers that are sealed to avoid contamination or loss of volatile substances during transportation. Once collected, the seawater samples are stored at room temperature, away from direct sunlight and heat, to preserve their integrity until they are ready for analysis. For metal analysis, 1 ml of concentrated HNO_3 is added to each sample to prevent precipitation of metals and ensure that the sample remains in a dissolved state (Figure 21). The acidified samples are stored in a sealed container until they are transported to the laboratory for further analysis. In the laboratory, the seawater samples are analyzed for trace metal concentrations.



Figure 21. Addition of concentrated HNO_3 to the seawater samples

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III.III. METAL ANALYSIS OF SEDIMENT

Sediment samples taken from the area where macroalgae are collected are taken from the top 0-5 cm layer of the sediment surface. Once collected, 1-2 ml of nitric acid is added to the samples, and they are labeled and stored at -20°C to preserve their integrity until further analysis.

Before metal analysis, sediment samples are removed from the freezer and the moisture is removed using blotting paper. They are then dried in an oven at 105°C for 24 hours. After drying, the sediment samples are passed through a motorized sieve shaker with varying mesh sizes, and particles with a size of 63 µm or smaller are used for determining element concentrations (Figure 22).

The small-sized samples designated for metal analysis are then combusted in a microwave digestion unit, after which they are analyzed using ICP-MS or AAS devices (Figure 23).



Figure 22. Sieving of dried sediment samples using a motorized sieve shaker

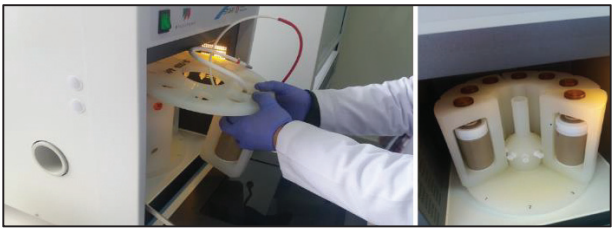


Figure 23. Microwave digestion unit

PART IV- GREENHOUSE

IV.I. CONSTRUCTION OF GREENHOUSE

The greenhouse is designed to be a minimum of 50 square meters (5.5 m x 9.09 m) and is enclosed (Figure 24 and Figure 25). The greenhouse dimensions are 5.5 m x 9.09 m, with a total area of 50 m². The minimum height at the center is 270 cm. It features two sliding doors, each with a minimum width of 1 meter, equipped with ventilation grilles (fitted with fly screens, openable and closable, metal, minimum size 45x45 cm).

The greenhouse structure is constructed using galvanized pipes. The main support poles, which are driven into the ground at each spring connection point, are made of 40 mm galvanized pipe, while the arch poles are made of 32 mm galvanized pipe. Ground connections are spaced appropriately to ensure wind resistance.

Along the side and top surfaces of the greenhouse, three rows of 32 mm galvanized pipe and five rows of evenly spaced galvanized wire (minimum 2 mm thickness) are installed, as shown in the illustration.



Figure 24. Visual representation of the greenhouse to be used in the study

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Figure 25. Installation of the greenhouse at SNU

The greenhouse has two doors (front and rear), made of metal profiles and covered with nylon sheeting. To increase structural stability, two vertical support pipes and scissor-type cross-braced pipes connected to a horizontal top pipe are used. The greenhouse covering is laid and anchored securely to prevent wind damage, with the edges buried in the soil. The galvanized pipes have a wall thickness of no less than 2.5 mm.

The plastic covering (greenhouse film) is a 36-month durable polyethylene material with UV, IR, EVA, AO, AV, and diffusion-resistant properties.

IV.II. PLANTS IN GREENHOUSE

Plants are cultivated in both pots and directly in soil inside the greenhouse. After the completion of the first and second trial periods, the pots are relocated to the soil area within the greenhouse. This strategy allows for more efficient use of the greenhouse space (Figure 26 and Figure 27). Analyzed soil is placed into the pots before planting.

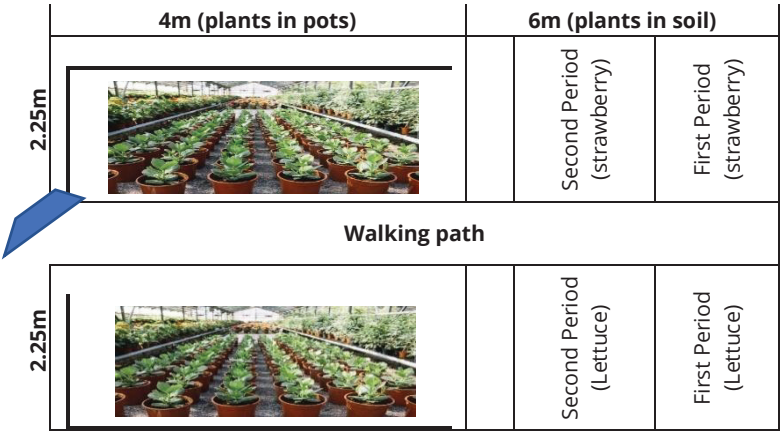


Figure 26. The layout plans of the pots and plants in the greenhouse (First and second periods)

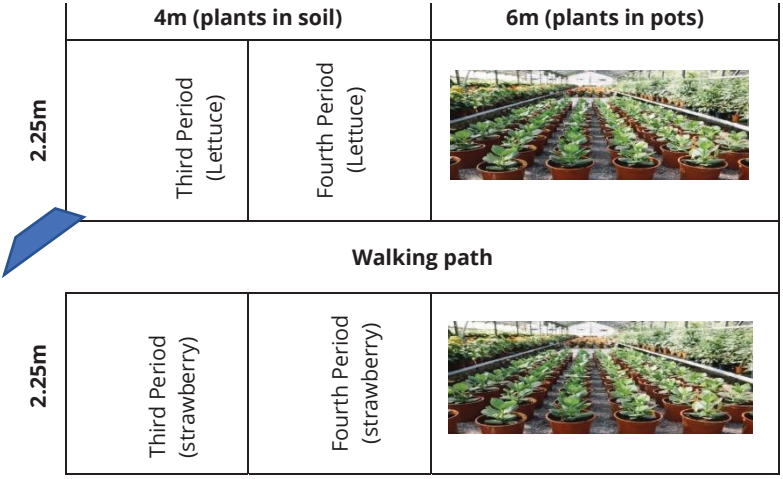


Figure 27. The layout plans of the pots and plants in the greenhouse (Third and fourth periods)

The study aims to apply liquid fertilizer, solid fertilizer (in both powder and pellet forms), and compost at three different concentrations: 1 g, 3 g, 5 g (for solids) and 1 ml, 3 ml, 5 ml (for liquids).

In addition, 2 pots are used for chemical fertilizer, and 2 pots serve as control in each trial period. Therefore, a total of 14 pots are required per plant species for each period.

Pot Allocation per Plant (per Trial Period)

- Liquid Fertilizer: 3 pots (1 ml, 3 ml, 5 ml)
- Solid Fertilizer (Powder): 3 pots (1 g, 3 g, 5 g)
- Solid Fertilizer (Pellet): 1 pot (3 g)
- Compost: 3 pots (1 g, 3 g, 5 g)
- Control and Chemical Fertilizer: 4 pots
- Total: 14 pots per plant species per period

IV.III. PLANT GROWTH TRIALS

Plant growth trials are carried out in two stages: pot trials and greenhouse trials. In the pot trials, controlled experiments are conducted using potted plants to examine the effects of macroalgae-based fertilizers on plant growth, yield, and nutrient uptake. These trials provide detailed insights under standardized conditions. In the greenhouse trials, semi-field experiments are performed under more natural conditions, allowing for the evaluation of fertilizer performance on a larger scale.

The pot is of the villa type and rectangular in shape, with a minimum capacity of 62 liters (dimensions: 38 x 86 x 30 cm). The material is UV-resistant and suitable for outdoor use. Each pot has a drainage hole and a base at the bottom.

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PART V - PLANT MATERIALS

In this research project, strawberry (*Fragaria* × *ananassa*) and lettuce (*Lactuca sativa L.*) were selected as representative plant materials.

Strawberry is a widely cultivated hybrid species from the Fragaria genus in the Rosaceae family, grown globally for its fruit. The selected variety is chosen for its broad ecological adaptability and short fruiting period. Strawberry seedlings to be planted in pots are supplied as bare-root plants, pruned to have two or three leaves and root lengths of approximately 10 cm (Figure 28).

Lettuce (*Lactuca sativa L*.) is an annual plant belonging to the Asteraceae family (Figure 29). This particular variety is selected for its ability to grow year-round and its short harvest cycle. Initially, lettuce seeds are germinated in vials. Once the seedlings develop 2–3 leaves, they are transplanted into pots. Relevant measurements are carried out during the plant's growth period.



Figure 28. Strawberry (*Fragaria x ananassa*) and strawberry seedlings

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Figure 29. Lettuce (*Lactuca sativa L.*)

V.I. VEGETABLE PERFORMANCE

The performance of vegetables grown with macroalgae-based fertilizers is evaluated through several key parameters. First, crop growth, yield, and quality attributes are measured and compared to those of vegetables grown with conventional fertilizers. This helps determine the effectiveness of macroalgae-based fertilizers in supporting plant development. Additionally, the nutrient content of the harvested vegetables is analyzed to assess how efficiently plants can uptake nutrients from these organic fertilizers. Finally, residue analysis is conducted to detect any potential contaminants or residues in the vegetables that may result from the application of macroalgae-based fertilizers.

V.II. PLANT ANALYSES

The study includes detailed analyses of plant growth, nutrient uptake, and residue levels in strawberry and lettuce plants.

a. Crop Growth and Yield: To evaluate growth and yield, various measurements and observations are performed on at least five samples

per treatment group. These include: plant height (above ground), leaf width and length, flower count (for strawberries), fruit count (for strawberries), plant body and leaf color, total plant length including roots, plant weight after removal from soil, as well as sensory evaluations such as color, taste, and smell. For lettuce, diameter measurements are also taken.

b. Nutrient Uptake: Nutrient analysis is conducted by sampling leaves during mid-growth. The following elements are analyzed: Nitrogen (N), Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg), Iron (Fe), Copper (Cu), Manganese (Mn), and Zinc (Zn).

For strawberries, the optimal sampling time is during active growth, before flowering and fruiting. Around 10–15 leaves (100–150 g) are collected randomly from different parts of various plants. Samples are transported under cold chain conditions.

For lettuce, leaf samples are collected 2–4 weeks before harvest. Again, 10–15 leaves (100–150 g) are taken from different areas of the plants and transported with cold chain preservation.

c. Residue Analysis: Heavy metal residue analysis is performed to detect the presence of metals such as Cu, Hg, Zn, Fe, Ca, ETC.

For strawberries, sampling occurs 3–7 days before harvest, when heavy metals are most likely to accumulate in leaves. Approximately 10–15 upper leaves are sampled randomly and preserved under cold chain conditions using hygienic storage materials such as ice boxes and protective packaging.

For lettuce, samples are collected 2–4 weeks before harvest when metal uptake is expected to be highest. 10–15 leaves from different sections of multiple plants are collected, totaling around 100–150 grams. These samples are also kept under strict cold chain and hygiene protocols.

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PART VI – SOIL MATERIALS

The soil analysis in the project focuses on assessing soil fertility and microbial activity by analyzing nutrient content and pH before and after applying macroalgae-based fertilizers. It also evaluates the impact of these fertilizers on microbial communities and nutrient cycling, offering insights into their influence on soil health.

VI.I. SOIL MATERIAL & ANALYSIS

The soil to be used in the experiment is sourced from the area surrounding the greenhouse established for the project (Figure 30). Before planting, the soil is prepared by passing it through a 1 cm sieve to remove any large particles. Soil samples are collected both before placing the soil in the pots and after the plants have completed their growth. Similarly, soil analysis is conducted both before and after planting for the plants grown in the greenhouse soil.



Figure 30. Soil material in greenhouse

Soil Fertility Assessment: Soil fertility is assessed by analyzing nutrient content and pH before and after applying macroalgae-based fertilizers. Samples (nearly 1000 grams) are taken from a depth of 0-20 cm, both before planting and after plant development. pH is measured with a pH meter, and organic matter analysis is conducted to evaluate the soil's nutrient status.

Soil Microbial Activity: The impact of macroalgae-based fertilizers on soil microbial communities and nutrient cycling is assessed. An increase in the total microbial mass indicates an increase in microorganisms, their biological activity, or improved environmental conditions for them. This increase generally has positive effects on agricultural productivity, environmental health, and ecosystem functioning. Microbial activity analyses are conducted for each group in each period, both before and after the experiment.

VI.II. IRRIGATION OF PLANTS

Irrigation is carried out using municipal water. An automatic irrigation system, connected to the outlet line of the water reservoir located outside the greenhouse, provides watering based on climatic conditions. The frequency of watering is determined according to the EC (Electrical Conductivity) levels (Strawberry: 1-2.5; Lettuce: 1.5-3), and the average pH is maintained at 6.

PART VII - FERTILIZERS

Three different types of fertilizers are prepared for the study: liquid fertilizer, compost, and solid fertilizer. Once prepared, the fertilizers are analyzed prior to their application to the plant samples.

VII.I. LIQUID FERTILIZER

The collected macroalgae (*Ulva* spp.) are soaked in tap water at +40 °C for 24 hours before proceeding with fertilizer production studies. The macroalgae are washed with running tap water until the salinity level drops below 1 PSU. Once the desired values are reached, the washing process is terminated, and the next stage is initiated.

Samples, prepared to be 0.5–1 cm in size after being extracted from the sea and pre-processed, will be mixed with pure water at a 1:1 ratio and placed into a fermentation tank (Figure 31). The fertilizer tank to be used has not been previously utilized. Simple mechanisms, such as plastic containers, have been employed. Therefore, minor modifications will be made to the existing fermentation tanks, including the installation of a locking mechanism to prevent air ingress.

The temperature inside the fermentation tank will be maintained between 22-26°C, and the pH should be 7. The pH will be monitored from a special compartment on the tank. If necessary, an acid such as KOH can be added to adjust the pH. A chemist can be consulted for precise pH adjustments. The mixer inside the fermentation tank will run for 2 minutes every 15 minutes. The fermentation period will last 30 days.

Once fermentation is complete, the liquid fertilizer will be transferred to bottles and mixed with pure water at a ratio of 1:5 (1 part fertilizer to 5 parts water). To stop the fermentation process, Sodium Metabisulfite (E223) (5.5 g/20 L) will be added to the bottles, along with Potassium Metabisulfite (2 g/10 L) as a mold and fungus inhibitor. For cleaning the fermentation tank, a mixture of Potassium Metabisulfite (20 g/10 L) and Citric Acid (10 g/10 L) will be used for disinfection. The mixture will be poured into the fermentation tank, and the lid will be loosely secured to allow gases to

escape slowly. After waiting for 30 minutes, the solution will be drained without the need for rinsing.



Figure 31. Liquid fertilizer tank

Liquid fertilizer is applied in three different concentrations (1 ml, 2 ml, and 3 ml). The first application is made immediately after the plants are planted in pots or soil. Subsequent applications are carried out at 15-day intervals until the plants complete their development. During each application, the liquid fertilizer is added to 1 liter of water at the specified concentrations (1 ml, 2 ml, and 3 ml) and applied to both pots and open soil.

VII.II. SOLID FERTILIZER

After being collected from the sea, the samples undergo preliminary processing, beginning with a drying process to reduce moisture content to below 10%. (Figure 32). They are then ground into a powder using a mill. From the resulting powder, fertilizers are produced in pellet form with diameters of 2.5 mm and 3 mm. Both the powder fertilizer and the pellets are purified separately.



Figure 32. Drying process in a climate-controlled chamber

Applications are made after the analysis is completed. Propionic Acid (95%) is used as a mold and fungus inhibitor for the solid fertilizer. After spraying, the fertilizer is kept in a closed container for 1 day. Then, the container lid is opened for another day, and the fertilizer is ready for use. A mixture of 20 ml water and 3 ml propionic acid is used per 1 kg of solid fertilizer.

The application of solid fertilizer starts just before planting. In other words, the fertilizer is applied first, and then the plant is planted. After applying the powder fertilizer, the soil is mixed to a depth of 10 cm. Fertilizer is applied at 15-day intervals until the plant completes its development. No fertilizer is applied to the control pot. The second pot receives 1 g of fertilizer, the third pot receives 3 g, and the fourth pot receives 5 g as a starting dose. For pellet application, the soil is mixed to a depth of 3-5 cm from the surface. The pellet is applied as a single dose in 1 pot and mixed into the soil. The application amount for the pellet is 3 g. Solid fertilizers are applied directly to the root areas of the plant.

VII.III. COMPOST FERTILIZER

After being collected from the sea and undergoing preliminary processing, the samples are chopped or cut to a size of 1–3 cm (Figure 33) and then placed in a fermentation device (Figure 34) to prepare compost using the 'Bokashi Method. The prepared compost is applied to the plants after analysis.

Propionic Acid (95%) is used as a mold and fungus inhibitor in the compost. After being applied via spraying, the compost is kept in a closed container for 1 day. The container lid is then opened for another day to allow the compost to air, making it ready for use. Compost fertilizers are applied starting immediately after planting. Fertilizer is then applied at 15-day intervals until the plant completes its development. No fertilizer is applied to the control pot. The second pot receives an initial dose of 1 g, the third pot receives 3 g, and the fourth pot receives 5 g of fertilizer.



Figure 33. Chopped macroalgae samples



Figure 34. Fermentation device

VII.IV. FERTILIZERS ANALYSIS

Before the fertilizers are applied to the plant samples, they undergo a thorough analysis. The analysis assesses various parameters for both liquid and solid fertilizers, as well as compost.

For the liquid and solid fertilizers, the following parameters are examined: moisture content, combustion loss, pH, electrical conductivity (EC), and the levels of essential nutrients such as nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu). Additionally, the presence of alginic acid, gibberellic acid, cytokinin, and auxin is also analyzed.

For the compost, the analysis includes moisture content, combustion loss, pH, EC, and the same essential nutrients (N, P, K, Ca, Mg, Fe, Mn, Zn, Cu). In addition, humic acid and fulvic acid are examined, along with alginic acid, gibberellic acid, cytokinin, and auxin.

VII.V. CHEMICAL FERTILIZER APPLICATION (15N, 15P, 15K)

In addition to macroalgae-based fertilizers, chemical fertilizers are also applied to each plant during the study. The chemical fertilizer used has a 15-15-15 composition (15% Nitrogen, 15% Phosphorus, and 15% Potassium).

For strawberries, 30 grams of chemical fertilizer are applied to the pot or soil just before planting. The soil is dug to a depth of 10 cm to plant the strawberries. Then, 15 grams of chemical fertilizer are applied every 30 days until the study is completed.

For lettuce, 25 grams of chemical fertilizer are applied immediately after planting. Subsequently, 15 grams of fertilizer are applied every 15 days until the lettuce plants have fully developed.

PART VIII – BIOPLASTICS

VIII.I. EXTRACTION PROCEDURE

The biomass, sized between 1-5 mm, is boiled with distilled water in a boiling flask using an electric heater for 30 minutes. The algal residue is then cooked for 30 minutes in a solution of $CaCl_2$ (0.5% W/V) in a boiling flask using an electric heater. After that, sodium chloride (0.5% W/V) is added to the algal biomass and boiled for 60 minutes. Finally, the algal residue is boiled with Na_2CO_3 (5% W/V) for 30 minutes with vigorous mixing. Sodium alginate is obtained by adding isopropyl alcohol or ethyl alcohol, followed by drying in an electric oven at 50°C for 28 hours.

VIII.II. BIOFILM PREPARATION

The preparation of biofilms begins by accurately measuring different quantities of algal residues, specifically ulvan or alginate, depending on the desired film properties. These biopolymers serve as the primary raw materials and are initially dissolved in distilled water to create a uniform solution. To enhance the flexibility, mechanical strength, and overall performance of the biofilms, various plasticizers such as glycerol, polyvinyl alcohol (PVA), polyethylene glycol (PEG), or other polymers commonly reported in the literature—such as cellulose derivatives—are incorporated into the solution at predetermined concentrations.

The mixture is subjected to continuous stirring using a magnetic or mechanical stirrer to ensure thorough dispersion of all components and to prevent any aggregation of the polymer chains. The stirring is carried out at controlled temperatures, which may vary depending on the specific protocol but generally ranges from room temperature to moderate heating, facilitating complete solubilization and homogenization of the solution.

Following this homogenization step, the prepared solution is carefully poured or cast onto clean petri dishes to form an even layer, which serves as the mold for the biofilm. These petri dishes are then placed in a drying oven maintained at 45°C, where the solvent (water) gradually evaporates

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over a 24-hour period. This drying process results in the formation of thin, uniform biofilms adhering to the surface of the dishes.

Once the films have completely dried, they are gently peeled off from the petri dishes to avoid any damage or tearing. To stabilize the physical properties and remove any residual moisture, the biofilms are conditioned for a minimum of 48 hours in a controlled environment with constant temperature and relative humidity. This conditioning step is crucial for ensuring reproducibility and reliability of the biofilm characteristics during subsequent testing or application (Figure 35).

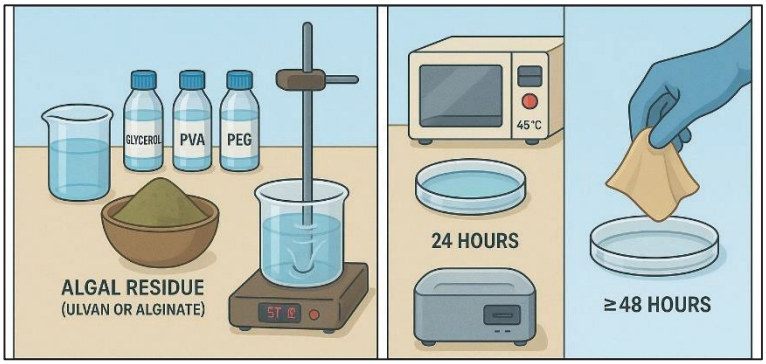


Figure 35. Biofilm preparation

VIII.III. CHARACTERISTICS OF THE FILMS

The characterization and evaluation of the produced bioplastics will be conducted through a series of rigorous tests designed to comprehensively assess their physical, optical, mechanical, morphological, thermal, and biodegradability properties.

These analyses are critical not only for determining the overall quality and performance of the fabricated seaweed-based bioplastics but also for enabling direct comparisons with conventional petroleum-derived plastics. By doing so, the study aims to demonstrate the technical feasibility and

environmental advantages of producing bioplastics from sustainable marine biomass, highlighting their potential contribution to the expanding green technology market.

Physical Properties: Key physical parameters are measured to understand the structural integrity and environmental durability of the films. Thickness is evaluated using precise micrometers or thickness gauges to ensure uniformity. Solubility tests examine the film's resistance to water and other solvents, indicating its suitability for various applications. Water vapor permeability (WVP) and water vapor transmission rate (WVTR) measurements are conducted to determine the film's barrier properties against moisture transfer; these properties are especially critical for packaging applications. Moisture content analysis measures the amount of water retained in the bioplastic, considered an important parameter affecting its mechanical and barrier properties.

Optical Properties: The visual quality and light interaction characteristics of the films are examined by measuring transparency, opacity, and light transmittance values at relevant wavelengths using spectrophotometry. These properties are important for applications that require specific optical clarity or light-blocking capabilities, such as food packaging or agricultural films.

Mechanical Properties: Mechanical tests are conducted using a universal testing machine to determine the tensile strength, elongation at break, and Young's modulus of the films. Tensile strength indicates the maximum stress the bioplastic film can withstand before breaking, elongation at break measures the film's flexibility and ductility, and Young's modulus reflects its stiffness and elasticity. These parameters are critical for assessing the durability and performance of the bioplastics in practical applications.

Morphological Study: Advanced analytical techniques are used to investigate the chemical composition and surface morphology of bioplastics. Fourier-transform infrared (FTIR) spectroscopy is employed to identify functional groups and chemical bonds, providing information about the molecular structure and interactions within the film. Field Emission Scanning Electron Microscopy (FE-SEM) reveals detailed surface

topography and microstructural features, while Atomic Force Microscopy (AFM) provides nanoscale surface roughness and texture information. X-ray diffraction (XRD) analysis is performed to determine the crystallinity and phase composition of the bioplastic materials, which influence their mechanical and thermal behavior.

Thermal Properties: Thermal Gravimetric Analysis (TGA) is performed to evaluate the thermal stability and decomposition patterns of the bioplastics. This technique measures weight loss as a function of temperature, providing valuable data on the temperature ranges within which the bioplastics maintain their integrity and the nature of thermal degradation processes.

Biodegradability: The environmental impact and sustainability of the bioplastics are evaluated through soil burial tests, where samples are buried in natural soil environments and monitored over time for degradation. This test provides empirical evidence of the biodegradation rate and the extent to which the bioplastic materials can be broken down by microbial activity, supporting their classification as eco-friendly alternatives to synthetic plastics.

VIII.IV. EXTRACTION METHOD

Alginate Extraction

Blender-mashed algal biomass (20 g) is mixed with 200 mL of distilled water in a beaker and heated at 50 °C for 30 min. The mixture is filtered, and the remaining algal residue is treated with 300 mL of calcium chloride solution (0.5%, w/v) at 50 °C for 30 min. After filtration, 300 mL of sodium chloride solution (0.5%, w/v) is added, and the mixture is heated at 50 °C for 60 min. The suspension is filtered again, and the algal residue is treated with 100 mL of sodium carbonate solution (5%, w/v) at 50 °C for 30 min under vigorous mechanical stirring. Ethyl alcohol is added to the filtrate to precipitate sodium alginate, and the product is dried in an electric oven at 50 °C for 28 h (Figure 36).

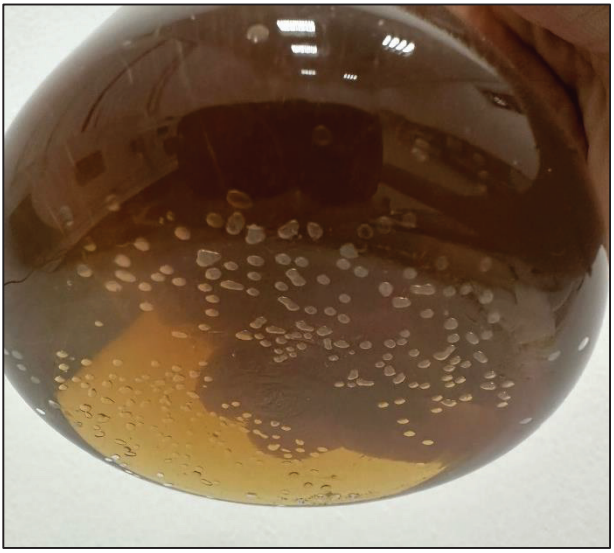


Figure 36. Alginate Extraction

Pre-treatment for Ulvan Extraction

Ulvan is extracted from green seaweed using the hot water extraction method. Prior to extraction, a pre-treatment is performed to remove lipids, pigments, and low-molecular-weight compounds such as phenols, free sugars, and amino acids. Washed and dried, blender-mashed algae are treated with ethyl alcohol using a Soxhlet extractor for 24 h. The supernatants are discarded, and the samples are dried again at 40 °C for 48 h before being stored for the hot water extraction process.

TOOLS & EQUIPMENT

AAS (Atomic Absorption Spectrophotometry): Used for analyzing metal concentrations.
Analytical Balance: Used to measure samples.
Atomic Force Microscopy (AFM): Used for analyzing the surface properties of biofilms at the nanoscale.
Automatic Irrigation System: Regulates water supply to crops.
Blotting Paper: Used for drying and removing excess water from cleaned samples.
Boiling Flask: Used for boiling algal biomass during processing.
Cold Chain Storage Equipment: Ice boxes and refrigerated transport to maintain sample integrity.
Concentrated HNO_3 (Nitric Acid): Used for acidifying samples to prevent precipitation of metals and preserve the sample's integrity.
Distilled Water: Used for rinsing collected samples to remove foreign materials.
EC Measurement Tools: Tools used to determine the EC levels to regulate irrigation frequency.
Electric Heater: Used for providing heat to the boiling flask.
Field Emission Scanning Electron Microscope (SEM): Used for studying the surface structure of biofilms.
Five Rows of Galvanized Wire: Used for reinforcing the greenhouse structure.
Formaldehyde-Seawater Solution (2–5%): Used for preserving macroalgae samples.
Fourier-Transform Infrared (FTIR) Spectroscopy: Used for analyzing the chemical structure of the produced bioplastics.
Freezer: Used for storing sediment samples to preserve their integrity before analysis.
Galvanized Pipes: Used for constructing the main framework and support system of the greenhouse, ensuring strength and durability against wind forces.
Greenhouse: Used for providing a controlled environment for plant growth, protected from external weather conditions.
ICP-MS (Inductively Coupled Plasma Mass Spectrometry): Used for analyzing the metal concentrations.
Isopropyl Alcohol or Ethyl Alcohol: Used for obtaining sodium alginate from algal residue.
Metal Profiles for Doors: Used for creating the frames for the doors of the greenhouse.
Microscope: Used for identifying macroalgae species through microscopic examination.
Microwave Digestion Unit: Used for digesting samples by applying heat and pressure to break down organic material and release metals for analysis.

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Mortar and Pestle: Used to grind dried macroalgae samples into fine powder for chemical analysis.
Motorized Sieve Shaker: Used for sieving dried sediment samples.
Multiparameter Device: Used for measuring in-situ physical parameters such as pH, temperature, salinity, and dissolved oxygen.
Oven: Used for drying samples.
Petri Dishes: Used to spread homogenized solutions of algal residue and plasticizers to prepare biofilms.
pH Meter: Used to measure the pH level.
Plastic Bags: Used for temporarily holding collected samples during transportation.
Plastic Covering (Greenhouse Film): Used for protecting plants inside the greenhouse from UV rays and environmental factors.
Plastic Jars: Used for transferring and preserving samples.
Plasticizers (Glycerol, Polyvinyl Alcohol, Polyethylene Glycol, Cellulose): Used for preparing biofilms and bioplastics from algal residues.
Pots: Used for growing plants inside the greenhouse.
Quadrate: Used for defining the sampling area for collecting macroalgae samples.
Sample Collection and Transport Containers: Used for ensuring proper storage and preservation during transportation.
Scanning Electron Microscope (SEM): Used for examining the surface morphology of the bioplastics.
Sealed Containers: Used to store acidified seawater samples during transportation to the laboratory.
Seawater Collection Containers: Used for collecting seawater samples.
Seedlings/Seeds: Used for planting.
Sensory Evaluation Tools: For evaluating color, taste, and smell of the vegetables.
Sliding Doors: Used for allowing easy access to the greenhouse and proper ventilation.
Sodium Chloride (0.5% W/V): Used for treating algal biomass in the process of making biofilms and bioplastics.
Soil Sampling Tools: Tools for collecting soil samples before and after plant growth.
Soil Sieving Equipment: For preparing the soil before planting by removing large particles.
Soil: Used for providing a growing for plants.
Soil-Burial Test: Used for assessing the biodegradability of bioplastics.
Spatula: Used for manually gathering macroalgae from the substrate.
Thermogravimetric Analyzer (TGA): Used for analyzing the thermal stability of the produced bioplastics.
Vertical Support Pipes and Cross-Braced Pipes: Used for enhancing the structural stability of the greenhouse.

Water Reservoir and Outlet Line: The water source for irrigation, located outside the greenhouse, connected to the irrigation system.
Weighing Scales: To measure plant weight after removal from soil.
X-ray Diffraction (XRD) Analysis: Used for determining the crystallinity and molecular structure of biofilms.

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Title of the Project

Research, Cleaning Methodologies, Economic Valorization and Pilot Application for Macroalgal Blooms

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