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Unveiling the Mineral Content of *Gracilaria* sp. Using X-Ray Fluorescence (XRF): The Potential of Red Algae in Environmental Monitoring

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Abstract

Background: *Gracilaria* sp. is a type of red algae that is easy to cultivate and produces agar. *Gracilaria* sp. thrives by absorbing nutrients from the water, and its thallus contains gel, which gives it a high water-binding capacity.
Aims: This study aims to identify the minerals absorbed by *Gracilaria* sp. using X-Ray Fluorescence (XRF) methods.
Result: According to the X-Ray Fluorescence (XRF) analysis, *Gracilaria* sp. contains 11 metal elements, with 6 primary elements including Potassium (K), Chlorine (Cl), Sulfur (S), Calcium (Ca), Silicon (Si), and Phosphate (P), as well as Iron (Fe), Magnesium (Mg), Bromine (Br), Manganese (Mn), and Strontium (Sr). Quantitatively, the dominant metal elements are Potassium (25.70%), Chlorine (22.90%), Sulfur (11.10%), Calcium (5.70%), Silicon (2.30%), and Phosphate (1.30%).
Conclusion: These findings indicate that *Gracilaria* sp. absorbs Potassium and Chlorine in significant amounts, with Sulfur being the next major element. Therefore, *Gracilaria* sp. can be used as an effective indicator for monitoring water quality and analyzing mineral distribution in aquatic ecosystems. *Gracilaria* sp. has potential applications in environmental remediation, particularly in addressing heavy metal pollution, and in the development of cultivation methods and practical uses in water quality management.

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1. Introduction

Indonesia, as an archipelagic country with 17,480 islands and a coastline of approximately 99,093 km, has about 70% of its area covered by oceans rich in various types of biological resources, including seaweed (Gardana *et al.,* 2007; Quatrin *et al.,* 2019). Seaweed is one of the abundant biological resources in Indonesian waters and belongs to benthic macroalgae that are attached to the bottom of aquatic environments (Haryanti *et al.,* 2012). Seaweed does not have roots, stems and leaves, which are called thallus. Seaweed is an aquatic organism that can suffer significant negative impacts from water pollution, such as heavy metal contamination from Lead (Pb) (Ishak *et al.,* 2020; Su *et al.,* 2015), which can be easily absorbed and accumulated by organisms (Handhani *et al.,* 2017). Seaweed can be used for phytoremediation. Phytoremediation is a method of bioremediation that utilizes plants to remove pollutants from contaminated water. The type often used is *Gracilaria* sp. (Komarawidjaja, 2005).

Gracilaria sp. belongs to the class of red algae (Rhodophyta), characterized by its red color caused by the pigment phycoerythrin. This red alga plays an important role in marine ecosystems, where it grows on hard seabeds such as rocks or coral reefs (Maftuch *et al.,* 2016; Prasedya *et al.,* 2019). *Gracilaria* sp. has a long, branched filament structure, with a thallus resembling fine red twigs, irregular branching concentrated at the base, and lateral branches extending like hair, measuring approximately 15-30 cm in length. This plant thrives by absorbing nutrients from the water and performing photosynthesis, thus its growth requires various physical and chemical factors (Rohman *et al.,* 2018). *Gracilaria* sp. is a red algae rich in gel, with a high water-binding capacity, and is one of the most cultivated and traded types of seaweed due to its high economic value and promising market prospects, both domestically and internationally (Komarawidjaja, 2005). The absorption process is also influenced by the shape of the thallus, both at the base and the branches of *Gracilaria* sp. (Tega *et al.,* 2023).

Naturally, the thallus at the base of *Gracilaria* sp. is attached to the substrate, resulting in a different absorption process compared to the tips of the thallus (Tega *et al.,* 2023). Appropriate water quality will have a positive impact on the productivity of aquaculture (Yunarty *et al.,* 2022). The success of seaweed cultivation is influenced by several factors such as the environment, seed quality, methods used, nutrient availability, and initial density or weight during maintenance (Agardh, 1837; Julyasih *et al.,* 2020). The cultivation of *Gracilaria* sp. can be conducted indoors. Indoor cultivation methods are more advantageous because water quality is easier to control, free from predators, and harvesting is simplified. The indoor method utilizes aquariums and seawater media with the addition of fertilizers as nutrient sources. The absorption of nutrients by the seaweed will enhance nutrient content and agar production (Adam et al., 2022; Ningrum et al., 2024).

The cultivation of *Gracilaria* sp. holds significant potential in the context of utilizing algae as a source of food and industry. This alga is known for its ability to absorb and accumulate various nutrients and minerals from the aquatic environment in which it grows. This process not only enhances cultivation productivity but can also affect the quality of the aquatic environment. However, the quality of *Gracilaria* sp. cultivation can be influenced by mineral contamination from polluted waters. To ensure that this alga is free from harmful elements that could jeopardize consumer health, it is important to analyze its mineral absorption. This analysis will provide a clearer picture of the mineral composition of the algae (Yanshin *et al.,* 2021). Although many studies have been conducted on mineral analysis techniques in various marine organisms, research on micro and macro mineral absorption by *Gracilaria* sp. remains limited. One of the mineral analysis techniques that can be used for *Gracilaria* sp. is X-Ray Fluorescence (XRF). This study aims to identify the mineral profile of *Gracilaria* sp. using XRF technology, with the hope of providing new insights into the nutritional aspects and safety of this algae. The data obtained from this analysis is expected to help optimize the cultivation of *Gracilaria* sp. and ensure that the cultivation results are safe and of high quality for consumption.

2. Methods

The research material used is *Gracilaria* sp., obtained from a cultivation pond at the National Research Agency (BRIN), North Lombok, West Nusa Tenggara, as shown in Figure 1. *Gracilaria* sp. is washed with water to remove dirt. Next, it is dried at a temperature of about 40-60°C. After the initial drying process, the samples are placed in an oven for further drying until all moisture is eliminated. Once the drying is complete, the samples are ground into a fine powder using a mortar grinder, and then sifted to obtain the *Gracilaria* sp. flour.

Figure 1. Sample Preparation of *Gracilaria* sp. Harvested *Gracilaria* sp. after 4 weeks (left); dried *Gracilaria* sp. (middle); *Gracilaria* sp. powder after grinding (right)

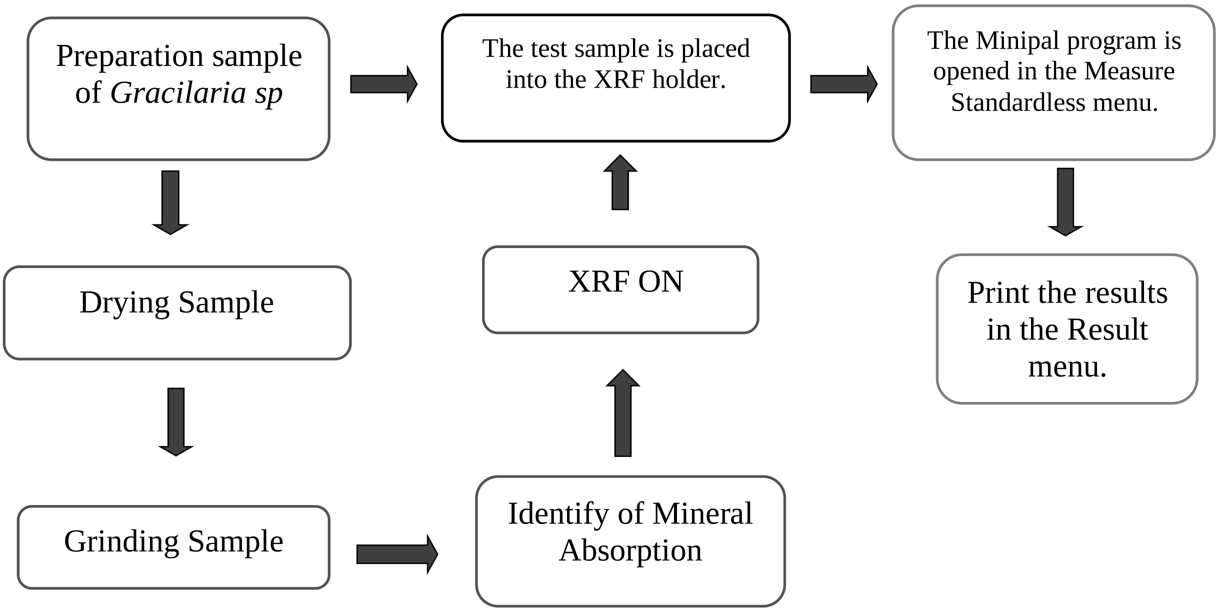

Figure 2. Procedure for Preparation and Identification of Minerals in *Gracilaria* sp.

Subsequently, the identification of absorbed minerals in the samples is carried out quantitatively using the X-Ray Fluorescence method (ED-XRF type) from Rigaku NexCG, as shown in Figure 3. The XRF testing procedure involves several steps. The first step is preparing the XRF device by turning it on, activating the HT On (X-Ray On), opening the Minipal program on the computer, and waiting approximately 10-15 minutes or until the device is fully operational. The next step involves preparing the powder and solid samples. This is done by using a holder fitted with a special plastic for XRF and placing the test sample into the holder. The final step is measurement, which involves inserting the sample into the XRF device, opening the Minipal program in the Measure Standardless menu, and entering the name of the sample to be measured in the Sample Ident and Measure fields (according to the sample order). After a few minutes, the analysis results will be displayed in the Result menu, and the desired results can be printed.

Figure 3. XRF with ED-XRF type

3. Results and discussion

The results of the X-Ray Fluorescence spectrophotometer analysis of *Gracilaria* sp. are presented in Table 1.

Table 1. Mineral Absorption in *Gracilaria sp.*

No	Compound Names	XRF Result
1	Kalium	√
2	Klorin	√
3	Sulfur	√
4	Kalsium	√
5	Silikon	√
6	Fosfat	√
7	Besi	√
8	Magnesium	√
9	Bromin	√
10	Mangan	√
11	Strontium	√

The quantitative analysis results of X-Ray Fluorescence for *Gracilaria* sp. identified 11 minerals. Based on the data obtained in the table, the 11 identified minerals include Calcium, Chlorine, Sulfur, Silicon, Phosphorus, Iron, Magnesium, Bromine, Manganese, and Strontium. Several metal minerals such as K, Ca, Fe, Mg, Mn, and Sr are present. In this case, Chlorine (Cl), Iron (Fe), Manganese (Mn), and Bromine (Br) are considered heavy metals and fall into the category of microminerals. *Gracilaria* sp. thrives by absorbing nutrients from the water and performing photosynthesis, so its growth requires various physical and chemical factors in the aquatic environment, such as water movement, temperature, salinity, nitrate, phosphate levels, and sunlight (Rohman *et al.*, 2018). The analysis results of XRF show the six dominant minerals contained in *Gracilaria* sp., as presented in Table 2.

Table 2. Percentage of Dominant Mineral Absorption in *Gracilaria sp.*

No	Chemical Coumpounds	Formula	Value
1	Kalium	K	25.70%
2	Klorin	Cl	22.90%
3	Sulfur	S	11.10%
4	Kalsium	Ca	5.70%
5	Silikon	Si	2.30%
6	Fosfat	P	1.30%

At the molecular level, the mineral composition in *Gracilaria* sp. is influenced by various factors, including the availability of nutrients in the water, salinity, environmental pH, growth conditions such as light and temperature, and interactions with microorganisms. Based on the data in Table 2, the three most abundant minerals in *Gracilaria* sp. are K at 25.70%, Cl at 22.90%, and S at 11.10%, followed by other minerals such as Ca at 5.70%, Si at 2.30%, and P at 1.30%. In this table, the values of K at 25.70%, Cl at 22.90%, and S at 11.10% show a very dominant presence compared to the others, especially K. Potassium (K) is an essential nutrient for plant growth and plays a crucial role in *Gracilaria* sp. Potassium also enhances the resilience of *Gracilaria* sp. to environmental stressors, including changes in salinity and nutrient deficiencies, and helps the algae combat pathogens. By regulating osmotic mechanisms, potassium contributes to water use efficiency, allowing the algae to adapt to fluctuations in the marine environment. Furthermore, in the cultivation of *Gracilaria* sp., adequate potassium availability can improve the quality and biomass size of the algae (Yunarty *et al.*, 2022). The quantitative XRF analysis identified this element in the samples, with the highest percentage (Adam *et al.*, 2018; Sartika *et al.*, 2024). The presence of K in the samples serves as a precursor for protein and carbohydrate formation, which are essential for the metabolism and energy production of the algae. Potassium aids in regulating osmotic pressure within the cells, maintaining the water balance that is vital for cellular stability (Yunarty *et al.*, 2022). In addition to potassium, seaweeds like *Gracilaria* sp. also contain various important micronutrients such as Chlorine (Cl), Iron (Fe), Manganese (Mn), and Bromine (Br). These micronutrients are required in small amounts but play crucial roles in various biological processes (Julyasih *et al.*, 2020; Maftuch *et al.*, 2016). A deficiency in any of these micronutrients can lead to growth delays and other physiological problems in the algae. Therefore, meeting the micronutrient requirements is essential to ensure optimal growth and development of *Gracilaria* sp. (Yunarty *et al.*, 2022).

In the research data, the micronutrients Chlorine (Cl), Iron (Fe), Manganese (Mn), and Bromine (Br) were identified in *Gracilaria* sp. with specific concentrations. Chlorine (Cl) was found in significant amounts, playing a role in the electrolyte balance and digestive functions of the algae. The substantial presence of chlorine in *Gracilaria* sp. highlights the importance of this element in supporting biochemical processes and the health of the algae, and it can be used as an indicator to assess the water quality in which the algae grow. Iron (Fe) is recorded as an essential micronutrient that supports metabolic processes in *Gracilaria* sp., including photosynthesis, respiration, and as a component of cellular proteins (Syam *et al.*, 2020). Additionally, Fe also assists in nitrogen metabolism and carbon assimilation, which are crucial processes in seaweed growth (Zainuddin & Nofianti, 2022). Iron is a vital nutrient for plants as it is needed for chlorophyll synthesis, plays a significant role in energy transfer, is part of several enzymes and proteins, and functions in respiration and plant metabolism, as well as being involved in nitrogen fixation (Zainuddin & Nofianti, 2022). Manganese (Mn) was also identified with important concentrations, functioning as an activator of several enzymes, including oxidases, peroxidases, dehydrogenases, and kinases that are involved in photosynthesis and nitrate reduction (Syam *et al.*, 2020). Fe acts as an enzyme catalyst in chlorophyll formation, while Mn is involved in enzyme activation (Syam et al., 2020). Meanwhile, Bromine (Br), although present in relatively smaller amounts, still plays a role in various important biological processes. The presence and levels of these micronutrients in *Gracilaria* sp. provide valuable insights into the health and nutritional status of the algae and can be used as indicators to assess water quality, manage aquatic environmental conditions, and apply environmental remediation, especially in addressing heavy metal pollution and developing cultivation methods. Plants capable of remediating by absorbing, accumulating, and releasing contaminants from the environment are known as hyperaccumulator plants (Ihsan *et al.*, 2015). These plants have an extraordinary capacity to absorb heavy metals and other contaminants at concentrations much higher than ordinary plants. One example of a hyperaccumulator is the seaweed *Gracilaria* sp. This seaweed not only absorbs heavy metals from polluted waters but also can accumulate significant amounts of nutrients and contaminants, making it an effective choice in phytoremediation techniques. With its significant ability to cleanse water from heavy metal pollution, *Gracilaria* sp. plays an essential role in environmental remediation efforts and water quality management.

4. Conclusion

The research results show that the mineral analysis of *Gracilaria* sp. using X-Ray Fluorescence (XRF) identified 11 minerals absorbed by the algae. Among these minerals, the six most dominant are Potassium (25.70%), Chlorine (22.90%), Sulfur (11.10%), Calcium (5.70%), Silicon (2.30%), and

Phosphate (1.30%). Potassium and Chlorine are the primary constituents in *Gracilaria* sp. *Gracilaria* sp. is potential to be used as environmental bioindicators with their ability to absorb pollutants and respond to environmental changes, for example in monitoring water quality, detecting pollution and evaluating ecosystem health.

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