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Original Research

Identifying the Environmental Factors that Determine the Agar Content of Seaweed *Gracilaria verrucosa* in Acid Sulfate Soil-Affected Brackishwater Ponds

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Abstract

This study was carried out with the aim to identify the characteristics and determine the direct or indirect effect of soil and water quality on the agar content of seaweed *Gracilaria verrucosa* in acid sulfate soil (ASS)-affected brackishwater ponds in South Sulawesi Province, Indonesia. The study was conducted in ponds of North Luwu and Sinjai Regencies. The soil quality is defined as the independent or exogenous variable; the water quality as an intermediate, dependent, or endogenous variable; as well as the agar content of seaweed as a dependent or endogenous variable. The influence of environmental factors was identified through path analysis applications. In general, the water quality can support the culture of seaweed in ponds. The results of the research showed that of the 17 soil quality variables analyzed, 2 variables were found, namely Fe content and pH_{KCl} which influenced the agar content of seaweed. Another result was that of the 9 water quality variables, and 2 variables were also found, namely salinity and NO_3^- content which influenced the agar content of seaweed. Soil Fe can reduce the agar content while soil pH_{KCl}, salinity, and water NO_3^- content can increase the agar content of seaweed in ASS ponds in South Sulawesi.

Keywords: acid sulfate soils, agar, brackishwater pond, path analysis, seaweed *Gracilaria verrucosa*

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Introduction

One of the culture fishery (aquaculture) commodities that are cultured in Indonesia both as a commodity for brackishwater ponds culture and mariculture, and which is harvested from the wild, is seaweed. The industrialization of national seaweed is a mandate of the Presidential Regulation of the Republic of Indonesia Number 33 of 2019, concerning the Road Map for the Development of the National Seaweed Industry for 2018-2021 (Presidential Regulation Number 33, 2019). Currently, Indonesia, which is blessed with ample land resources and potential for seaweed culture, has become one of the main producers of seaweed in the world with wet seaweed production reaching 11.6 million tonnes in 2016, mostly for the seaweed *Eucheuma* sp and *Gracilaria* sp. For comparison, in 2016, the world's wet seaweed production was around 30.0 million tonnes, so Indonesia contributed nearly 40% of the world's total seaweed production [1].

Seaweed culture is an alternative activity that is environmentally sound, contributes to overcoming poverty, and will leverage the economy of the population of coastal areas, which amounts to 60% of Indonesia's total population (Presidential Decree Number 33, 2019). Seaweed is an important economic macroalgae for the production of agar and carrageenan.

From the red algae (Rhodophyta) class such as seaweed *Gracilaria verrucosa*, *Gracilaria confervoides*, *Gracilaria gigas*, *Gracilaria lichenoides*, *Gracilaria crassa*, *Gracilaria blodgettii*, *Gracilaria arcuata*, *Gracilaria taenioides*, *Gracilaria eucheumoides*, and many more. *G. verrucosa* (Hudson) Papenfuss 1950 is an aquaculture commodity that has long been cultured in Indonesian ponds, including in South Sulawesi Province. In 2015, South Sulawesi was ranked first as a producer of seaweed in Indonesia, where 880,885 tonnes (76.12% of 1,157,234 tonnes of total production) came from the ponds of South Sulawesi [2]. Until 2019, ponds in South Sulawesi reached an area of 108,464 ha spread across 19 regencies/cities from 24 regencies/cities. Total pond production in South Sulawesi in 2019 was 1,205,507 tonnes, of which 903,077 tonnes (74.91% of total pond production) came from the culture of seaweed *G. verrucosa* on the east coast of South Sulawesi, namely East Luwu, North Luwu, Luwu, Wajo, Bone, and Sinjai Regencies as well as Palopo City (FMS of South Sulawesi Province, 2020). Seaweed *G. verrucosa* is well known for its economic benefits in the production of agar, human food, pet food, pharmaceuticals, biofuels, cosmetics, and fertilizers [3-6]. Seaweed *G. verrucosa* produces primary metabolites in the form of hydrocolloid compounds called agar. Agar is a complex mixture of polysaccharides composed of two major fractions: agarose and agaropectin. Agarose, a neutral polymer, and agaropectin, a charged, sulfated polymer [7]. Agarose, the gelling fraction, is a neutral linear molecule essentially free of sulfates, consisting of chains of repeating alternate units of β-1,3-linked-D-galactose and α-1,4- linked 3,6 anhydro-L-galactose units [8]. Agaropectin, the non-gelling fraction, is a sulfated polysaccharide (3% to 10% sulfate), composed of agarose and varying percentages of ester sulfate, D-glucuronic acid, and small amounts of pyruvic acid [7, 8]. The proportion of these two polymers varies according to the species of seaweed. Agarose normally represents at least 70% of the natural agar. The biosynthesis of agar in seaweed is influenced by genetic variation, stage of development, and environmental conditions [9]. Gioele et al. [10] and Al-Haddad [11] state that the main factors that influence the yield and quality of agar are the type of algae, physiological factors, location, season, and environmental conditions. Various environmental conditions such as nutrient status, light intensity, salinity, and water temperature can affect the agar content of seaweed [12, 13]. This shows that the environmental factor in which seaweed is cultured is one of the important factors that can affect the agar content of seaweed.

Ponds on the east coast of South Sulawesi are generally dominated by acid sulfate soils (ASS) and ASS is associated with peat soils [14]. ASS is defined as a soil or sediments containing pyrite (FeS_2). When ASS is constructed into ponds, FeS_2 oxidation occurs which can cause a decrease in soil pH of less than 3.5. The next impact is that the solubility of toxic elements such as aluminum (Al), iron (Fe), and manganese (Mn) becomes higher which can kill cultured organisms and absorb phosphate (PO_4^{2-}) into an unavailable form that can reduce soil and water fertility ponds. In this case, the soil quality of ASS can affect pond water quality and seaweed performance, including its agar content. Soils are a major production factor in aquaculture because it affects water quality, biological processes, and pond engineering [15]. Although water quality management is considered one of the most important aquaculture factors, there is a lot of evidence that pond bottom soil conditions and the exchange of substances between soil and water greatly affect water quality [16, 17]. The quality of pond bottom soil and the processes that occur in the pond bottom soil and the relationship between the soil and water of the pond are very important for the growth of fish or shrimp [17-19] or seaweed. Generally, seaweed *G. verrucosa* is cultured in ponds using the broadcast method, so that it is not only in the water body but also in direct contact with the pond bottom soil. Water quality problems in ponds are frequent and can stem from the effects of low soil quality, such as low pH and alkalinity water in the soil acid, low dissolved oxygen content as a result of the amount of oxygen needed for the decomposition of organic matter in soil and the presence of reduced compounds such as NO_2^-, hydrogen sulfide (H_2S), Fe, and Mn which is produced by anaerobic microorganisms in the soil [20]. However, there is no detailed information regarding the causal relationship between soil quality and or water quality in affecting the agar content of seaweed in ASS ponds. Therefore, this research aimed to identify the characteristics of the pond environment and to analyze the causal relationship between the variables of soil quality and water quality and agar content of seaweed *G. verrucosa* in ASS-affected brackishwater ponds of South Sulawesi.

Experimental

Research Locations

The research was conducted in brackishwater pond areas on the east coast of South Sulawesi Province, Indonesia namely North Luwu and Sinjai Regencies (Fig. 1). Analysis of the soil quality, water quality, and agar content of seaweed was carried out respectively at the Soil, Water, and Biotechnology Laboratories of the Research Institute for Coastal Aquaculture (RICA) or recently known as the Research Institute for Coastal Aquaculture and Fisheries Extension (RICAFE) in Maros Regency, South Sulawesi.

Data Collection

The survey method was applied in this research to obtain data on the quality of soil and water and the agar content of seaweed. Before measuring and taking soil, water, and seaweed samples, interviews are conducted with pond farmers to know the age of the seaweed from the ponds they manage. Determination of ponds as sampling points based on purposive sampling method, namely ASS ponds with seaweed between 40-50 days old. As stated by Fethi and Ghedifa [12], the age of seaweed greatly affects the agar content and other compositions, and seaweed that is around 45 days old has a high agar content.

In the selected ponds, measurements and samples of soil and water were taken. Measurement and soil sampling were carried out at a depth of 0-0.20 m in three places in each pond which were then composited. The soil quality variables that are measured *in situ* are pH_F with pH-meter (HANNA, HI 8424, Romania) [21], pH_{FOX} with pH-meter (HANNA, HI 8424, Romania) [21], and redox potential with redox-meter (HANNA, HI 8424, Romania). pH_F is soil pH measured directly in the field and pH_{FOX} is soil pH measured in the field after being oxidized with hydrogen peroxide (H_2O_2) 30%. Redox potential indicates how oxidized or reduced (anaerobic) the soil is, as well as the electron availability in the soils. For the analysis of other soil quality variables, soil samples that have been taken using a soil auger are placed in a plastic bag and then put in a cool box filled with ice according to the instructions of Ahern et al. [21]. Because all soil samples were ASS, the soil samples were dried at 80-85°C for 48 hours [21]. After drying, the soil samples were pulverized by pounding them on a porcelain mortar and sieving with a 2.0 and 0.5 mm sieve hole size. Soil quality analyzed at the laboratory included pH of KCl extract (pH_{KCl}) [22], pH of H_2O_2 extract (pH_{OX}) [23], sulfur peroxide (S_P) [24], sulfur extracted with KCl (S_{KCl}) [25], sulfur peroxide oxidizable (S_{POS}, S_P - S_{KCl}) [21], Titratable Actual Acidity or previously known as Total Actual Acidity in 1 M KCl titrated to pH 5.5 (TAA), Titratable Peroxide Acidity or previously known as Total Potential Acidity in 1 M KCl peroxide digest titrated to pH 5.5 (TPA), Titratable Sulfidic Acidity or previously known as Total Sulfidic Acidity (TSA, TPA - TAA), pyrite [26-28], Al, Fe, organic matter, and PO_4^{2-} [29].

Fig. 1. Research locations of seaweed Gracilaria verrucosa in acid sulfate soil-affected brackishwater ponds in North Luwu and Sinjai Regencies, South Sulawesi Province, Indonesia.

Water quality characteristics are known by *in situ* measurements in each selected pond, including temperature, salinity, pH, and dissolved oxygen with Hydrolab® (Minisonde, Water Quality Multiprobe, United States of America). Other water quality characteristics are identified by water sampling and preserved according to instructions of APHA [30]. The water quality analyzed included nitrite (NO_2^-) with the colorimetric method, nitrate (NO_3^-) with the cadmium reduction method, PO_4^{2-} with the ascorbic acid method, sulfate (SO_4^{2-}) with the turbidimetric method, and Fe with the phenanthroline method [30].

In each selected pond, seaweed samples were taken. The agar content of seaweed is known by performing seaweed extraction following the method of Jayasinghe et al. [31]. A total of 10 g of dried seaweed samples were immersed in 0.25% chlorine solution for 3 × 24 hours at room temperature. The seaweed is then rinsed and soaked in freshwater for three hours, and then soaked using 0.1% sulfuric acid for 15 minutes. After soaking with sulfuric acid, the samples were immersed for 15 minutes in freshwater followed by extraction of agar by cooking with 500 mL of distilled water until the remaining water became one-third of the portion. The resulting agar is filtered and poured into trays for sun drying. Dry examples are weighted and the agar content is measured based on the formula from Kumar and Fotedar [32]:

$$Ca = \frac{Wa}{Dw} \times 100\% \quad (1)$$

where:
Ca: content of agar
Wa: weight of agar
Dw: dry sample weight.

Data Analysis

The pond soil quality is the independent, or exogenous, variable in this research; the intermediate, dependent, or endogenous variable is the pond water quality, while the agar content of seaweed is the dependent, or endogenous, variable.

Descriptive statistics are used to determine the minimum, maximum, average, and standard deviation values of existing data. The correlation coefficient was determined to detect multicollinearity symptoms, namely the symptom of correlation between variables of soil quality and water quality of ponds. A high correlation coefficient indicates a symptom of multicollinearity between variables and then one of them is selected by selecting a variable that is easier to determine in the field. The path analysis model used is recursive. The path diagram model is based on the relationship paradigm between variables, while the structural equation path diagram follows the path diagram model. The enter method is used to calculate the regression equation. The re-correlation coefficient is used to determine the correlation between selected exogenous variables and between selected intermediate variables and the combination of exogenous and intermediate variables. The adjusted coefficient of determination (R^2) test was used to determine the magnitude of the exogenous variable explaining the intermediate variable and the combination of the exogenous and intermediate variables explaining the dependent variable. The F test is used to test the existence of a linear relationship between exogenous variables and between exogenous and intermediate variables. The t-test is used to determine the influence of exogenous variables on the intermediate variables individually or partially and the magnitude of the influence of exogenous and intermediate variables on the dependent variables partially as well. The significance level is set at 0.05. All data were analyzed with the help of the Statistical Product and Service Solution (SPSS) program version 25. The magnitude of the influence of other variables outside the model is determined by calculating the path coefficient or standardized regression coefficient or "beta" (ρ) which shows an error using a formula [33]:

$$\rho = \sqrt{1 - R^2} \quad (2)$$

where:
ρ: path coefficient
R^2: coefficient of determination.

Determination of the amount of influence, both direct effect, indirect effect, and the total effect of exogenous variables on endogenous variables are calculated based on the instructions of Kwan and Chan [33] and Harris and Gleason [34]. The path analysis results diagram was created with the help of the Analysis of Moment Structures (AMOS) program version 22.

Results and Discussion

Characteristics of Environmental Factors and Agar Content of Seaweed

Soil Quality

As previously explained, the soil types found in the brackishwater pond areas of the east coast of South Sulawesi are dominated by ASS, so the soil quality variables analyzed are typical soil quality variables or specific characteristics for ASS (Table 1). The E_h variable is the most important variable to determine the electrochemical properties of inundated soil, such as pond soil. The average E_h of pond soil on the east coast of South Sulawesi is negative, namely -299.4 mV which indicates that the soil is in a reduced condition. Based on the opinion of Husson [35], ASS ponds on the east coast of South Sulawesi are classified as highly reduced, because the E_h value ranges from -100 to -300 mV. This is a result of the pond being completely inundated because it is used for seaweed culture. When the soil is flooded, oxygen is pushed out and the decomposition process takes place in an anaerobic state. When the entire soil pore space is filled with water, the availability of oxygen in the soil is drastically reduced. After the oxygen is depleted, the soil E_h will range from +400 to –300 mV, as was found in this research.

The pH_F and pH_{FOX} of ASS ponds in South Sulawesi averaged 6.803+0.450 and 1.856+0.933. The Δ pH (pH_F - pH_{FOX}) value varied from 1.97 to 5.88 with an average of 4.968. This shows that the potential for the acidity of the ASS ponds in South Sulawesi is relatively varied and high. The Δ pH of ASS is usually greater than 4.0 [14].

Table 1. Descriptive statistics of soil quality used for seaweed Gracilaria verrucosa culture in acid sulfate soil-affected brackishwater ponds of South Sulawesi Province, Indonesia (n = 47).

Variables	Minimum	Maximum	Average	Standard Deviation
E_h (mV)	-386	-123	-299.4	65.5
pH_F	5.26	7.82	6.803	0.450
pH_{FOX}	0.49	5.39	1.856	0.933
pH_F - pH_{FOX}	1.97	5.88	4.968	0.794
pH_{KCl}	2.90	6.53	4.696	1.083
pH_{OX}	0.68	2.63	1.305	0.409
TPA (mole H^+/tonnes)	57.0	913.0	426.24	177.95
TAA (mole H^+/tonnes)	0	52.0	14.07	16.08
TSA (mole H^+/tonnes)	26.0	913.0	358.59	203.34
S_{KCl} (%)	0.0242	2,4425	0.39125	0.38673
S_P (%)	0.3883	4,4047	2,31613	0.96569
S_{POS} (%)	0.2688	3,3260	1.67155	0.74361
FeS_2 (%)	0.161	4,0759	1,84006	0.79024
Fe (ppm)	1,210	5,920	4,592.0	1,238.0
Al (ppm)	138.5	1,194.5	442.83	264.47
Organic matter (%)	1.39	25.29	9.215	5.653
PO_4^{2-} (ppm)	0.0491	8.2305	3.03936	1.99363

In ASS characterized by FeS_2 content, one of the sources of acidity is sulfur (S). Oxidized FeS_2 will produce sulfuric acid (H_2SO_4) and ferrous sulfate (iron (II) sulfate, 2 valence iron, $FeSO_4$) which when reacting with water releases ferric sulfate (iron (III) sulfate, 3 valence iron, $Fe_2(SO_4)_3$) which furthermore when it is re-oxidized it will produce H_2SO_4. As an important source of acidity in ASS, the S measured in the form of soil S_{POS} has been used by [27] to determine lime requirements for ASS. The available data shows that the soil S_{POS} content is quite high, which means that it requires high lime as well. The high lime requirement is also indicated by the high content of toxins such as Al and Fe. The high Al and Fe content in ASS ponds of South Sulawesi resulted in a relatively lower soil PO_4^{2-} content. This is due to soil Al and Fe which can cause PO_4^{2-} to become unavailable. In low pH soils, PO_4^{2-} is strongly bound by Al and Fe in the form of insoluble $AlPO_4$ or $FePO_4$ [36].

The soil organic matter content in ASS ponds of South Sulawesi varied from 1.39 to 25.29%. This also indicates that the pond soil in South Sulawesi is classified as organosol soil or peat soil. Peat soil is soil characterized by an organic matter content that exceeds 20% [37]. Common ASS is also found in association with peat soil as found in this research.

Soil quality is generally less supportive for brackishwater commodities. However, high seaweed production can still be obtained in ASS ponds with certain soil conditions. It has been previously reported that high seaweed production in ASS ponds was found in soil with a pH_F greater than 6.5, a pH_{FOX} greater than 4.0, Δ pH less than 2.5, and S_{POS} less than 1.00% [38]. It was also said that of the 12 soil quality variables analyzed, only 1 soil variable had a significant effect on the production of seaweed *G. verrucosa*, namely pH_{FOX} in ASS ponds in Luwu Regency. Furthermore, García-Poza et al. [5] stated that the quality including the agar content of seaweed *Gracilaria* sp is influenced by the quality of the pond bottom soils.

Water Quality

The water temperature in ASS ponds of South Sulawesi was 32.576+1.969ºC (Table 2). High water temperatures are generally measured in ponds where the water is very shallow (less than 0.2 m). The water temperature of 25-30ºC is a good temperature for seaweed culture [39]. Temperature directly affects seaweed in the process of photosynthesis, metabolic processes, and the reproductive cycle. Cebrián-Lloret [40] states that temperature is one of the environmental factors that have a significant effect on the agar content of seaweed where the higher the temperature the higher the agar content of seaweed. Water temperature and salinity extremes may affect the quality of seaweed *G. verrucosa* agar products [12].

Table 2. Descriptive statistics of water quality used for seaweed Gracilaria verrucosa culture in acid sulfate soil-affected brackishwater ponds of South Sulawesi Province, Indonesia (n = 47).

Variables	Minimum	Maximum	Average	Standard Deviation
Temperature (°C)	29.98	37.86	32.576	1.969
Salinity (ppt)	8.22	27.18	17.558	3.511
pH	6.31	9.32	8.342	0.501
Dissolved oxygen (mg/L)	3.16	9.85	5.372	1.823
NO_3^- (mg/L)	0.0002	0.0607	0.01618	0.01266
NO_2^- (mg/L)	0.0001	0.0259	0.00536	0.00489
PO_4^{2-} (mg/L)	0.0026	0.1360	0.02600	0.0801
SO_4^{2-} (mg/L)	12.37	50.85	24.021	8.693
Fe (mg/L)	0.0004	0.0795	0.03443	0.01880

On the east coast of South Sulawesi, many rivers have freshwater sources, so the salinity of pond water can range from 8.22 to 27.18 ppt with an average of 17.558 ppt. This is a condition that is quite favorable, because water salinity is relatively easy to adjust according to the requirement of seaweed culture, especially in locations close to freshwater and saltwater sources. As reported by Sahu et al. [41] previously seaweed *G. verrucosa* grows the fastest at salinity of 25 ppt. Rahim [42] stated that seaweed *G. verrucosa* grows well at salinity between 18 and 30 ppt.

The tolerance limit for aquatic organisms to pH varies and is influenced by many factors, including dissolved oxygen, temperature, alkalinity, and the presence of anions and cations as well as the type and stage of the organism. The optimum water pH for the culture of seaweed *Gracilaria* sp is 7.0-8.0 [43]. Table 2 shows that in general, ASS ponds in South Sulawesi whose water pH is classified as supporting the growth of seaweed. There have been many studies on the physiological and biological responses of red algae such as seaweed *Gracilaria* sp to abiotic factors such as light intensity, pH, salinity, and temperature [44, 45]. pH is responsible for the agar content of seaweed *Gracilaria manilaensis* [46]. At low pH, agar can lose its function where depolymerization can occur [47].

Dissolved oxygen comes from air diffusion and results from the photosynthesis of chlorophyll organisms that live in water and are needed by organisms to oxidize the nutrients that enter their bodies. Brackishwater bottom soil is an important component of oxygen dynamics in ponds. Organic fertilizer, feces, and dead plankton settle to the bottom soil and are decomposed by bacteria creating an oxygen demand. Water dissolved oxygen in ASS ponds of South Sulawesi ranged from 3.16 to 9.85 mg/L with an average of 5.372 mg/L. In this case, dissolved oxygen in pond water is classified as suitable for the culture of seaweed in ponds. The culture of seaweed *Gracilaria* sp in ponds requires dissolved oxygen of at least 4 mg/L and optimally in the range of 4-6 mg/L [43]. The availability of dissolved oxygen in the media is used by macroalgae for respiration. Respiration in plants is the process of taking O_2 to break down organic compounds into CO_2, H_2O, and energy. The daily aeration period influences the production of seaweed *Gracilaria* sp, but it does not affect the agar content [48].

The growth of seaweed is more influenced by the nutrient content in the water, the growth of seaweed increases with the increase in nutrient content in the water, but on the other hand, the hydrocolloid content decreases in the high nutrient content [49]. Ganesan et al. [50] stated that seaweed *Gracilaria* sp can contain different agar contents depending on the seed, age, culture method, nutrients, and harvest time. Nutrients are one of the factors that influence the growth of seaweed. Nutrients such as N can be absorbed by seaweed in the form of ammonium (NH_4^+) and NO_3^- [51, 52]. From Table 1, it can be seen that the NO_3^- content of pond water in South Sulawesi ranges from 0.0002 to 0.0607 mg/L with an average of 0.01618 mg/L. According to Rejeki et al. [53], seaweed requires a NO_3^- content range of 0.9-3.5 mg/L.

NO_2^- is an intermediate form between ammonia (NH_3^+) and NO_3^- (nitrification) and between NO_3^- and nitrogen gas (denitrification). The NO_2^- content of pond water in South Sulawesi ranges from 0.0001 to 0.0259 mg/L with an average of 0.00536 mg/L which is classified as not high. The NO_2^- content in the waters is relatively small because it is immediately oxidized to NO_3^-. Natural waters contain about 0.001 mg/L of NO_2^- and should not exceed 0.06 mg/L [54].

It has also been reported by Stedt et al. [55] which states that there is a strong relationship between the NH_4^+ content and the growth of seaweed *Gracilaria* sp. Seaweed *Gracilaria* sp absorbs NH_4^+ faster than NO_3^- [56], however, seaweed *Gracilaria* sp production is not affected by the form of N if the N content is above the minimum content in water [57]. Seaweed *Gracilaria tikvahiae* and *Gracilaria cornea* have the same growth

Table 3. Agar content of seaweed Gracilaria verrucosa cultured in acid sulfate soil-affected brackishwater ponds of South Sulawesi Province, Indonesia

Content of Agar (%)				Location (Regencies)	References
Minimum	Maximum	Average	Standard Deviation		
nd	nd	25.81	5.54	East Luwu	Rahim (2017)
nd	nd	15.20	1.70	Luwu	Makmur & Mulyaningrum (2018)
nd	nd	22.19	2.45	Sinjai	Rosmiati et al. (2019)
5.75	28.74	nd	nd	Luwu	Syam et al. (2020)
13.17	36.55	22.882	5.427	North Luwu and Sinjai (n = 47)	This research

Note: nd: no data available

rate when N is given in the form of NH_4^+ and NO_3^-, or both forms simultaneously [58]. It was also reported that in seaweed *Gracilaria foliifera* and *Gracilaria pacifica* the absorption rate of NH_4^+ was higher than that of NO_3^- [56]. Seaweed *Gracilaria lemaneiformis* can absorb NH_4^+, NO_3^-, and NO_2^- respectively 68.44%; 23.03%; and 13.04% for 24 hours [58].

In water, the element P is not found in free form as an element, but in the form of dissolved inorganic compounds (orthophosphate and polyphosphate) and organic compounds in the form of particulates. PO_4^{2-} is a form of P that can be used by plants [59]. Based on the PO_4^{2-} content, waters are classified into three, namely: waters with low fertility, which contain PO_4^{2-} 0-0.02 mg/L; waters with moderate fertility, which contain PO_4^{2-} 0.021-0.05 mg/L; and waters with high fertility levels, which contain PO_4^{2-} 0.051-0.10 mg/L [60]. Based on these criteria, it shows that the quality of pond water in South Sulawesi (Table 2) is classified as medium and high fertility. The agar content of seaweed *Gracilaria* sp seems to increase with decreasing P under a high N supply [61]. Seaweed can grow optimally with a PO_4^{2-} range from 0.051 to 1.00 mg/L [62]. Thus, the PO_4^{2-} content of ASS pond water in South Sulawesi is low for seaweed culture.

The range of Fe content in ASS pond water of South Sulawesi is from 0.0004 to 0.0795 mg/L with an average of 0.01880 mg/L. Resz et al. [63] stated that the Fe content in natural waters ranges between 0.05 and 0.20 mg/L. Also, aquatic plants and algae can absorb metals such as Fe. Furthermore, Du et al. [64] stated that algae can absorb Fe three times more than other metals. Aquatic plants and algae have a higher tolerance than fish and shrimp to the presence of metals [65].

Many factors have been reported to have significant effects on the agar content of seaweed *Gracilaria* sp, such as temperature, N, light, salinity, and stage of development [12]. Thus, the variables of pond water quality can affect the agar content of seaweed in ASS ponds. Water quality variables also have an important role in the formation of agar, especially temperature and light intensity, where the content of seaweed increases in conditions of increased light intensity [66]. This can be understood because temperature and light intensity greatly affect the photosynthetic process of seaweed which produces carbohydrates in the form of agar [67].

Agar Content of Seaweed

The level of agar content is influenced by the species of seaweed, environmental conditions, and the age of harvest. Environmental factors in which seaweed *Gracilaria* sp grows, namely the content of the main nutrients in the form of N and P, temperature, salinity, dissolved oxygen, and pH can affect the metabolism and synthesis of pigments, proximate, and agar [68]. The difference in the content of seaweed *Gracilaria* sp agar that is cultured at each location is thought to be due to physiological processes and ecological adaptations such as different light intensity, pH, and salinity at each location so that it affects the nutrient absorption process [69]. Agar is a polysaccharide that accumulates in the cell walls of gel-producing seaweed which is influenced by the season [9].

The agar content of seaweed *G. verrucosa* reared in ASS ponds of this research which were harvested at the age between 40 and 50 days varied from 13.17 to 36.55% with an average of 22.882+5.427% (Table 3). This agar content of seaweed is relatively the same as that reported by Rosmiati et al. [70] which obtained 22.19+2.45% agar content from seaweed cultured in the ponds of Sinjai Regency (Table 3), one of the locations of this research. Seaweed *G. verrucosa* is classified to be of high quality if the agar content is high, which is greater than 25% [71]. In general, it can be seen that the content of seaweed *G. verrucosa* in various pond locations in South Sulawesi is relatively the same. When compared with the criteria of NSA [71], it shows that the agar content of seaweed cultured in ASS ponds of South Sulawesi is still classified as high agar content. The agar

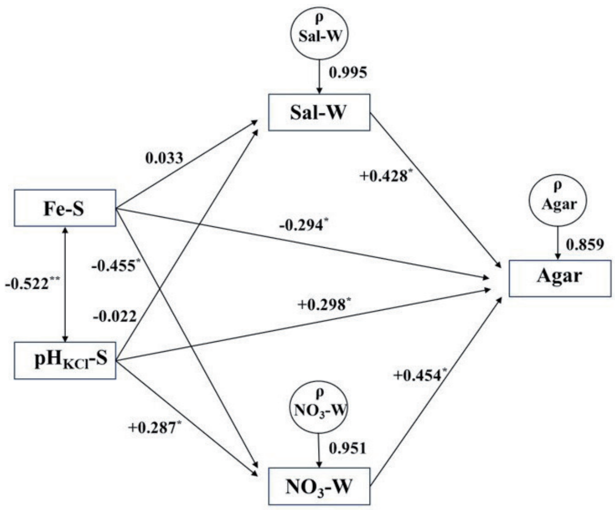

Fig. 2. Diagram of the results of the path analysis of the influence of soil quality on water quality and the agar content of Gracilaria verrucosa seaweed in acid sulfate soil-affected brackishwater ponds of South Sulawesi Province, Indonesia. (Soil Fe (Fe-S), Soil pHKCl (pHKCl-S), Water salinity (Sal-W), Water NO3- (NO3-W), Path coefficient (ρ), P <0.01 (**), P <0.05 (*)).

content varies between 6 and 71% in various references, but 20% is a common value [72].

Relationship between Environmental Factors and Agar Content of Seaweed

It has been previously explained that soil quality variables are independent or exogenous variables in this research. Of the 17 variables of soil quality (Table 1) analyzed, it turned out that only 2 variables significantly affected the agar content of seaweed in the ASS ponds of South Sulawesi, namely soil Fe (Fe-S) and soil pH_{KCl} (pH_{KCl}-S) (Fig. 2, Table 4). Soil Fe has a significant effect (P<0.05) with a direct effect of -0.294, an indirect effect of -0.014, and a total effect of -0.308 on the agar content of seaweed (Fig. 2). Soil Fe which affects the agar content of seaweed has a significant effect (P <0.05) on water quality, namely water NO_3^- (NO_3-W) (Fig. 2).

Other soil quality variables, namely soil pH_{KCl} have a significant effect (P <0.05) with a direct effect +0.298, the indirect effect -0.130, and the total effect +0.168 on the agar content of seaweed (Fig. 2, Table 4). Soil pH_{KCl} also had a significant effect (P <0.05) on water quality, namely water NO_3^- (Fig. 2).

Of the 9 water quality variables which are intermediate, dependent, or endogenous in this research, it turns out that only 2 variables affect the agar content of seaweed in ASS ponds of South Sulawesi, namely salinity (Sal-W) and water NO_3^- (NO_3-W) content (Fig. 2, Table 4). Salinity and NO_3^- of water also had a significant effect (P<0.05) (Fig. 2, Table 4) with a direct effect of +0.428 and +0.454 respectively on the agar content of seaweed.

Fe which is abundant in ASS ponds [14] is needed by plants including as a constituent of cytochromes and chlorophyll and plays a role in enzyme systems and electron transfer in the photosynthesis process [73]. Fe, as one of the essential mineral elements for algae growth, plays a very important role in physiological processes such as plant photosynthesis, respiration, N fixation, protein synthesis, and nucleic acids [74]. Fe is also a metal needed by algae the most compared to other metals [64]. The availability of elemental Fe limits algae growth and its deficiency has been shown to limit phytoplankton growth [75]. However, based on Fig. 2 shows that the effect of soil Fe is negative on the agar content of seaweed, which means that an increase in soil Fe content will reduce the agar content of seaweed. Soil Fe content, which ranged between 1,210 and 5,920 ppm with an average of 4,592.0+1,238.0 ppm, is thought to be quite high which could have an impact on reducing the agar content of seaweed. In anaerobic and acid soils, high concentrations of Fe^{2+} ions can cause Fe toxicity due to excessive absorption of Fe [76]. Excess Fe can be very toxic, as it reacts with oxygen and catalyzes the production of free radical species. Fe poisoning in aquatic plants such as rice (*Oryza sativa*) occurs because excess Fe can interfere with metabolic processes and cause damage to plants, characterized by the presence of rusty leaves (bronzing symptoms), stiff and brown structures, and underdeveloped root systems [77]. It was also said that, generally, the concentration of Fe in the range of 1,000-2,000 ppm in the soil could affect lowland rice production. It has been confirmed by Sinaga et al. [78] that excess Fe can cause poisoning in rice plants, even though it is classified as resistant to conditions of high Fe content, some of them are poisoned at soil Fe content of 500 ppm.

Another effect of soil Fe on reducing the agar content of seaweed is thought to be through its effect on the availability of PO_4^{2-}. P is a limiting factor for primary productivity in brackishwater ponds. P is an essential element for higher plants and algae such as seaweed, so this element becomes a limiting factor for seaweed and greatly affects the level of aquatic productivity. P is another important nutrient because it participates in the formation of biomolecules such as nucleic acids, proteins, and phospholipids [79]. However, its most important role is in the energy transfer mediated by adenosine triphosphate (ATP) and other high-energy compounds present in photosynthesis and respiration [80]. P can stimulate the growth and photosynthetic rate of some algae and increase the production of agar and carrageenan of seaweed [81]. The PO_4^{2-} content in ASS is generally very low because it is bound by Fe, making it unavailable to aquatic organisms including seaweed that need it. In ASS, PO_4^{2-} is strongly bound by Fe in the form of insoluble $FePO_4$ [36]. Thus, the higher the soil Fe content, the lower the PO_4^{2-} availability for seaweed, which will harm the agar content of the seaweed.

Soil pH is considered the "major variable" in soil chemistry because of its large impact on chemical reactions involving essential nutrients, phytotoxic elements, and pollutants. Both directly and indirectly, pH affects the solubility of nutrient elements, which determines their biological availability and mobility. From Fig. 2 it can be seen that the type of soil pH that affects the agar content of seaweed is pH_{KCl}. pH_{KCl} shows the pH value of the soil after the hydrogen ion (H^+) in the uptake complex is pushed out and into the soil solution by other cations so it is also called potential soil pH. pH management is very important for environmental management, including the pond environment in increasing seaweed production and the quality of seaweed such as its agar content. From Fig. 2 it can be seen that an increase in pH_{KCl} also causes an increase in the agar content of seaweed. Soil pH_{KCl} which ranges between 2.90 and 6.53 with an average of 4.696 is classified as low, so an increase in pH_{KCl} has an impact on increasing the availability of nutrients needed to increase the agar content of seaweed. Multiple regression analysis shows that of the 12 soil quality variables analyzed, only 1 soil variable has a significant effect on the production of seaweed *G. verrucosa*, namely pH_{FOX} in ASS ponds in Luwu Regency [38] and then said by Mustafa et al. [82] that pH_{KCl} and pH_{FOX} have the same data pattern in ASS ponds in Mamuju Regency (West Sulawesi Province). The absorption of nutrients will generally be optimum at neutral soil pH because, at this pH level, nutrients will dissolve easily in water. Soil pH also affects the performance of bacteria and fungi as decomposers of organic matter as well as binding of N elements, where bacteria generally thrive at a pH of 5.5 or more, while fungi can live at all levels of soil acidity. Therefore, an increase in pH will lead to higher nutrient solubility which can be utilized by plants including seaweed, which also has an impact on increasing the agar content of seaweed. It has been reported that environmental factors such as pH and nutrients affect the agar content of seaweed [68, 83]. Soil pH also affects the performance of bacteria and fungi as decomposers of organic matter as well as binding of N elements, where bacteria generally thrive at pH 5.5 or more while fungi can live at all levels of soil acidity. Therefore, an increase in pH will lead to higher nutrient solubility which can be utilized by seaweed which also has an impact on increasing the agar content of seaweed.

Fig. 2 shows that there is a negative correlation (-0.522) between pH_{KCl} and Fe content of the soil. In this case, increasing pH_{KCl} will decrease soil Fe content. In ASS ponds, the low pH causes Fe solubility to be high, and conversely, the increase in ASS pH causes the soil Fe solubility to be below. Increasing pH causes ferric ion (Fe^{3+}) to oxidize and bind to hydroxide to produce $Fe(OH)_3$ which is insoluble and settles (precipitation) and forms a reddish color on the soil [36, 84]. On the other hand, soil Fe can also affect soil pH, where the high solubility of Fe causes an increase in soil acidity or a decrease in soil pH. The high solubility of Fe is accompanied by a hydrolysis process so that many H^+ ions are released into the soil solution which causes the soil to become acidic. This is made clear by Ifansyah [85] that pH, Al, Fe, and P are soil properties that are related to one another.

The indirect effect of Fe on the agar content of seaweed is not only through salinity but also through the water NO_3^- content. The direct effect of Fe on NO_3^- is negative, in this case, the higher the Fe content, the lower the NO_3^- content (Fig. 2). According to Dariah et al. [86] removing or reducing toxicity from Fe, increasing pH, and providing Ca elements for plants through liming can increase the availability of several nutrients such as N, P, and molybdenum (Mo). This indicates that the increase in Fe content can reduce the availability of the element N so that the Fe in ASS ponds has a negative effect (-0.022) on the water NO_3^- content (Fig. 2, Table 4).

The best salinity to produce the highest agar content of seaweed (24.80±2.96%) was obtained at a salinity of 25 ppt [87]. It has been reported by Anton [88] that the highest agar content of seaweed *G. verrucosa* was obtained at a salinity of 20 ppt compared to the salinity

Table 4. Direct, indirect, and total effects of each correlation in the path analysis for soil quality, water quality, and agar content seaweed Gracilaria verrucosa in acid sulfate soil-affected brackishwater ponds of South Sulawesi Province, Indonesia.

Correlation in Path Analysis	Direct Effect	Indirect Effect	Total Effect
Fe-S → Agar	-0.294	-0.014	-0.308
Fe-S → Sal-W	-0.033	-0.126	-0.159
Fe-S → NO_3-W	-0.455	-0.042	-0.497
pH_{KCl}-S → Agar	+0.298	-0.130	+0.168
pH_{KCl}-S → Sal-W	-0.022	+0.128	+0.106
pH_{KCl}-S → NO_3-W	+0.287	+0.135	+0.422
Sal-W → Agar	+0.428	+0.003	+0.431
NO_3-W → Agar	+0.454	+0.085	+0.539

Note: Soil Fe (Fe-S), Soil pHKCl (pHKCl-S), Water salinity (Sal-W), Water NO3- (NO3-W).

of 10, 15, 25, and 30 ppt. From Table 1 it can be seen that the salinity of the ASS ponds water in South Sulawesi ranges between 8.22 and 27.18 ppt with an average of 17.558+3.511 ppt which indicates that the salinity is low compared to the optimum salinity required for seaweed agar production. This causes salinity to affect the agar content of seaweed in the ASS ponds of South Sulawesi. Based on Fig. 2, it can be seen that the effect of salinity is positive (+0.428) on the agar content of seaweed, which means an increase in salinity to a certain salinity will increase the agar content of seaweed. The high content of agar at a salinity of 20-25 ppt is thought to be due to the osmotic pressure of the extracellular fluid and the intracellular fluid of seaweed in optimal conditions so that the diffusion process of nutrient absorption takes place well and effectively. The stress caused by low or high salinity will affect the distribution of sugar as a result of the photosynthesis process which is responsible for maintaining ion balance. This assumption is in line with the statement of Synytsya et al. [89], that the process of forming agar in the seaweed cell walls takes place with the development of cells that absorb a lot of nutrients and through the process of photosynthesis which is converted into various polysaccharides including agar. Salinity affects the agar content of seaweed in the ionic equilibrium of the cell because of the negative charge in the polysaccharide cation-anion balance [47]. The physiological effects of growing seaweed include biochemical and biophysical processes that convert simple molecules of CO_2 and H_2O into sugars, amino acids, and polysaccharides, where the result of this process will increase the agar content of seaweed [5].

Nutrients are one of the factors that influence the growth of seaweed. Nutrients such as N can be absorbed by seaweed in the form of NH_4^+ and NO_3^- [51, 90]. NO_3^- is the main form of N in natural waters and is the main nutrient for plant growth including seaweed. From Table 1, it can be seen that the NO_3^- content of pond water in South Sulawesi ranges from 0.0002 to 0.0607 mg/L with an average of 0.01618+0.01266 mg/L. NO_3^- is a compound that plays a role in supporting the growth of seaweed. According to Wang et al. [56], seaweed requires a range of NO_3^- content of 0.9-3.5 mg/L. These results show that the water NO_3^- content in the ASS ponds of South Sulawesi is relatively low, so an increase in the NO_3^- content of water will increase the agar content of seaweed. From Fig. 2 it can be seen that the NO_3^- content has a positive effect (+0.454) on the agar content of seaweed. When the N content is high in water, the photosynthesis of seaweed is diverted toward protein synthesis instead of polysaccharide synthesis [91]. This supports the previous explanation that the low N water content in ASS ponds of South Sulawesi indicates a photosynthetic process that can increase the agar content of seaweed. The N content will affect the synthesis and content of chlorophyll algae [92, 93]. Algae chlorophyll will have a direct effect on the photosynthesis process and indirectly will support the growth rate and agar content of seaweed. The ability of plants to reduce and accumulate NO_3^- into the form of NO_2^- (NO_3^- to NO_2^-) and from NO_2^- to NH_4^+ is due to the presence of the nitrate reductase enzyme which is very helpful in the formation of plant chlorophyll [94]. Plant chlorophyll plays an important role in the process of photosynthesis, where photosynthesis itself indirectly affects the agar content of seaweed.

The causal relationship between the variables of soil quality and water quality and agar content of seaweed in the ASS ponds of South Sulawesi is shown in the model presented in Fig. 2. The magnitude of the influence of other variables outside the model is known from the path coefficient (ρ) which shows the value of ρ = 0.859 for the agar content of seaweed, which means that the influence of soil and water quality has a causal relationship to the agar content of seaweed is 0.141. In this case, the influence of soil and water quality as a causal relationship to the agar content of seaweed in ASS ponds of South Sulawesi was 14.1%, the remaining 85.9% was influenced by other factors that were not detected in this research.

It appears that ASS ponds are problematic for the production of seaweed agar. This requires the management of ASS in the form of increasing soil pH, decreasing soil Al and Fe content, and increasing soil fertility. Increasing pH and decreasing Al and Fe content can be done through remediation, both remediation in the form of drying, flooding, and flashing the soil as well as in the form of liming. The principle of remediation through drying, flooding, and flashing the soil is drying the soil to oxidize FeS_2, flooding to dissolve and neutralize acidity or reduce further acid production, and flushing to remove oxidation products and minimize reserves of soil Al and Fe elements which ultimately also increase pH soil [95–98]. Another form of remediation in the form of liming can be carried out to increase the pH and reduce toxic elements such as Al and Fe and the elements that cause soil acidity that remain in the soil. Increasing soil fertility can be done by applying fertilizers containing N and P. Through the application of remediation and increasing soil fertility, it is hoped that the growth and agar content of seaweed *G. verrucosa* seaweed in ASS ponds of South Sulawesi can increase.

Conclusions

Brackishwater ponds on the east coast of South Sulawesi are dominated by ASS with low pH, high potential of acidity, low macronutrient content, and high toxic content. In general, water quality can support seaweed *Gracilaria verrucosa* culture in ponds. Of the 17 soil quality variables analyzed, it turned out that only 2 variables were soil Fe content and soil pH_{KCl} which determine the agar content of seaweed, while from 9 water quality variables, it turned out that only 2 variables were salinity and water NO_3^- content which determine the agar content of seaweed in ASS ponds of South Sulawesi. Soil Fe can reduce the agar content, while soil pH_{KCl}, salinity, and water NO_3^- content can increase the agar content of seaweed in ASS ponds. Soil improvement through remediation either in the form of drying, flooding, and flushing or in the form of liming and through fertilization with fertilizers containing N and P to increase the agar content of seaweed cultured in ASS-affected brackishwater ponds in South Sulawesi.

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Author Contributions

Akhmad Mustafa conceived and designed the research. Akhmad Mustafa, Erna Ratnawati, Mudian Paena, and Kamariah carried out the research. Akhmad Mustafa, Admi Athirah, and Andi Indra Jaya Asaad performed the acquisition of data, data analyses, and interpretation. Akhmad Mustafa wrote and reviewed the manuscript with support from Admi Athirah, Mohammad Syaichudin, and Imam Taukhid. All authors discussed the results and contributed to the final manuscript. Major contributors are Akhmad Mustafa and Admi Athirah, while member contributors are Erna Ratnawati, Mudian Paena, Kamariah, Andi Indra Jaya Asaad, Tarunamulia, Ruzkiah Asaf, Mohammad Syaichudin, and Imam Taukhid.

Conflict of Interest

The authors declare no conflict of interest.

References

1. FERDOUSE F., HOLDT S.L., SMITH R., MURÚA P., YANG Z. The global status of seaweed production, trade and utilization. FAO Globefish Research Programme. **124** (120), **2018**.
2. MMAF. Regulation of the Minister of Marine Affairs and Fisheries of the Republic of Indonesia Number 75/ Permen-KP/2016 about General Guidelines for Grow-out of Tiger Shrimp (*Penaeus monodon*) and Whiteleg Shrimp (*Litopenaeus vannamei*). Available online: https://bkipm.kkp.go.id/bkipmnew/public/files/regulasi/75-permen-kp-2016-ttg-pedoman-umum-pembesaran-udang-windu.....pdf. **2016**.
3. WELLS M.L., POTIN P., CRAIGIE J.S., RAVEN J.A., MERCHANT S.S., HELLIWELL K.E., SMITH A.G., CAMIRE M.E., BRAWLEY S.H. Algae as nutritional and functional food sources: Revisiting our understanding. Journal of Applied Phycology. **29** (2), 949, **2017**.
4. GOMEZ-ZAVAGLIA A., PRIETO LAGE M.A., JIMENEZ-LOPEZ C., MEJUTO J.C., SIMAL-GANDARA J. The potential of seaweeds as a source of functional ingredients of prebiotic and antioxidant value. Antioxidants. **8** (9), **2019**.
5. GARCÍA-POZA S., LEANDRO A., COTAS C., COTAS J., MARQUES J.C., PEREIRA L., GONÇALVES A.M.M. The evolution road of seaweed aquaculture: cultivation technologies and the Industry 4.0. International Journal of Environmental Research and Public Health. **17** (18), 6528, **2020**.
6. FARGHALI M., MOHAMED I.M.A., OSMAN A.I., ROONEY D.W. Seaweed for climate mitigation, wastewater treatment, bioenergy, bioplastic, biochar, food, pharmaceuticals, and cosmetics: A review. Environmental Chemistry Letters. **21** (1), 97, **2023**.
7. GU Y., CHEONG K.L., DU H. Modification and comparison of three *Gracilaria* spp. agarose with methylation for promotion of its gelling properties. Chemistry Central Journal. **11** (1), 1, **2017**.
8. CIANCIA M., MATULEWICZ M.C., TUVIKENE R. Structural diversity in galactans from red seaweeds and its influence on rheological properties. Frontiers in Plant Science. **11** (9), 1, **2020**.
9. LEE W.K., LIM Y.Y., LEOW A.T.C., NAMASIVAYAM P., ABDULLAH J.O., HO C.L. Factors affecting yield and gelling properties of agar. Journal of Applied Phycology. **29** (3), 1527, **2017**.
10. GIOELE C., MARILENA S., VALBONA A., NUNZIACARLA S., ANDREA S., ANTONIO M. *Gracilaria gracilis*, Source of agar: A short review. Current Organic Chemistry. **21** (5), 380, **2017**.
11. AL-HADDAD S.H. Algae: Potential biotechnological source in the Arabian Gulf. African Journal of Biotechnology. **19** (4), 183, **2020**.
12. FETHI M., GHEDIFA A. BEN. Optimum ranges of combined abiotic factor for *Gracilaria gracilis* aquaculture. Journal of Applied Phycology. **31 (**5), 3025, **2019**.
13. WIDYARTINI D.S., HIDAYAH H.A., INSAN A.I. Diversity and distribution pattern of bioactive compound potential seaweed in Menganti Beach, Central Java, Indonesia. Biodiversitas. **24** (2), 1125, **2023**.
14. MUSTAFA A., RATNAWATI E., CHAIDIR UNDU M. Characteristics and management of brackishwater pond soil in South Sulawesi Province, Indonesia. IOP Conference Series: Earth and Environmental Science. **564** (1), **2020**.
15. DRÓŻDŻ D., MALIŃSKA K., KACPRZAK M., MROWIEC M., SZCZYPIÓR A., POSTAWA P., STACHOWIAK T. Potential of fish pond sediments compost as organic fertilizers. Waste and Biomass Valorization. **11 (**10), 5151, **2020**.
16. BOYD C.E., D'ABRAMO L.R., GLENCROSS B.D., HUYBEN D.C., JUAREZ L.M., LOCKWOOD G.S., VALENTI W.C. Achieving sustainable aquaculture:

Historical and current perspectives and future needs and challenges. Journal of the World Aquaculture Society. **51** (3), 578, **2020**.
17. BOYD C.E., QUEIROZ J.F. The role and management of bottom soils in aquaculture ponds. Infofish international. **2** (22), **2014**.
18. BOYD C.E., LI L. Reactions between pond bottom soil, water. Glob. Aquacult. Advocate. Available online: https://www.globalseafood.org/advocate/reactions-between-pond-bottom-soil-water/, **2011**.
19. LIU X., SHAO Z., CHENG G., LU S., GU Z., ZHU H., SHEN H., WANG J., CHEN X. Ecological engineering in pond aquaculture: a review from the whole-process perspective in China. Reviews in Aquaculture. **13** (2), 1060, **2021**.
20. MUSTAFA A., ATHIRAH A. Path analysis application in determining the effect of soil and water quality on the total production of brackishwater ponds in Demak Regency, Central Java Province. Jurnal Kelautan Nasional. **9** (2), 65, **2014**.
21. AHERN C.R., BLUNDEN B., SULLIVAN L.A., MCELNEA A. . Soil sampling, handling, preparation and storage for analysis of dried samples. Acid sulfate soils laboratory methods guidelines, Available online: http://www.nrme.qld.gov.au/land/ass. **2004**.
22. MCELNEA A., AHERN C. Peroxide pH (pHOX), titratable peroxide acidity (TPA) and excess acid neutralising capacity (ANCE). In: Acid Sulfate Soils Laboratory Methods Guidelines pp. 1-8, **2004**.
23. MCELNEA A., AHERN C. KCl extractable pH (pHKCl) and titratable actual acidity (TAA). In: Acid Sulfate Soils Laboratory Methods Guidelines pp. 1-3, **2004**.
24. MCELNEA A., AHERN C. Sulfur-peroxide oxidation method. In: Acid Sulfate Soils Laboratory Methods Guidelines pp. 1-2. Indooroopilly, Queensland: Queensland Department of Natural Resources, Mines and Energy. **2004**.
25. MCELNEA A., AHERN C. Sulfur 1M KCl extraction (SKCl). In: Acid Sulfate Soils Laboratory Methods Guidelines pp. 2-3, Indooroopilly, Queensland: Queensland Department of Natural Resources, Mines and Energy. **2004**.
26. AHERN C.R., RAYMENT G.E. Codes for acid sulfate soils analytical methods. In: C.R. Ahern, B. Blunden, & Y. Stone, Acid Sulfate Soils Laboratory Methods Guidelines pp. 3.1-3.5. Wollongbar, NSW: Acid Sulfate Soil Management Advisory Committee. **1998**.
27. AHERN C.R., MCELNEA A., BAKER D.E. Acid neutralizing capacity methods. In: C.R. Ahern, B. Blunden, & Y. Stone, Acid Sulfate Soils Laboratory Methods Guidelines pp. 6.1-6.4. Wollongbar, NSW: Acid Sulfate Soil Management Advisory Committee. **1998**.
28. AHERN C.R., MCELNEA A., BAKER D.E. Peroxide oxidation combined acidity and sulfate. In: C.R. Ahern, B. Blunden, & Y. Stone, Acid Sulfate Soils Laboratory Methods Guidelines pp. 4.1-4.17. Wollongbar, NSW: Acid Sulfate Soil Management Advisory Committee. **1998**.
29. MENON R.G. Soil and Water Analysis: A Laboratory Manual for the Analysis of Soil and Water. Sumatera Selatan: Palembang: Proyek Survey O.K.T., **1973**.
30. APHA. Standard Methods For the Examination of Water and Wastewater 20th ed.; Washington, D.C.: American Public Health Association. Available online: https://www.standardmethods.org/doi/book/10.2105/SMWW.2882, **2005**.
31. JAYASINGHE P.V.P., RANAWEERA. Effect of extraction methods on the yield and physiochemical properties of polysaccharides extracted from seaweed available in Sri Lanka. Poultry, Fisheries & Wildlife Sciences. **4** (1), 1, **2016**.
32. KUMAR V., FOTEDAR R. Agar extraction process for *Gracilaria cliftonii* (Withell, Millar, & Kraft. 1994). Carbohydrate Polymers. **78** (4), 813, **2009**.
33. KWAN J.L.Y., CHAN W. Comparing standardized coefficients in structural equation modeling: A model reparameterization approach. Behavior Research Methods. **43** (3), 730, **2011**.
34. HARRIS J.E., GLEASON P.M. Application of path analysis and Structural Equation Modeling in nutrition and dietetics. Journal of the Academy of Nutrition and Dietetics. **122** (11), 2023, **2022**.
35. HUSSON O. Redox potential (E_h) and pH as drivers of soil/plant/microorganism systems: A transdisciplinary overview pointing to integrative opportunities for agronomy. Plant and Soil. **362** (1–2), 389, **2013**.
36. PENN C.J., CAMBERATO J.J. A critical review on soil chemical processes that control how soil ph affects phosphorus availability to plants. Agriculture (Switzerland). **9** (6), 1, **2019**.
37. KAZEMIAN S. Organic soils and peats. In: Encyclopedia of Engineering Geology; pp. 691-695, **2018**.
38. MUSTAFA A., SAMMUT J. Dominant factors affecting seaweed (*Gracilaria verrucosa*) production in acid sulfate soils-affected ponds of Luwu Regency, Indonesia. Indonesian Aquaculture Journal. **5** (2), 147, **2010**.
39. KUMAR Y.N., POONG S.W., GACHON C., BRODIE J., SADE A., LIM P.E. Impact of elevated temperature on the physiological and biochemical responses of *Kappaphycus alvarezii* (Rhodophyta). PLoS ONE. **15** (9), 1, **2020**.
40. CEBRIÁN-LLORET V., MARTÍNEZ-ABAD A., LÓPEZ-RUBIO A., MARTÍNEZ-SANZ M. Sustainable bio-based materials from minimally processed red seaweeds: Effect of composition and cell wall structure. Journal of Polymers and the Environment. **31** (3), 886, **2023**.
41. SAHU N., GANESAN M., ESWARAN K. Inter- and intra-generic grafting in seaweeds in the Indian coasts. Current Science. **99** (2), 235, **2010**.
42. RAHIM A.R. Seaweed Cultivation Techniques *Gracillaria verrucosa* in pond Ujungpangkah District, Gresik East Java using broadcast method. International Journal of Environment, Agriculture and Biotechnology. **3** (4), 1305, **2018**.
43. COKROWATI N. Seaweed Culture Technology. Semarang: MAI Publishing. **2016**.
44. SKRIPTSOVA A.V., NABIVAILO Y.V. Comparison of three gracilarioids: Growth rate, agar content and quality. Journal of Applied Phycology. **21** (4), 443, **2009**.
45. KAMAL M., ABDEL-RAOUF N., ALWUTAYD K., ABDELGAWAD H., ABDELHAMEED M.S., HAMMOUDA O., ELSAYED K.N.M. Seasonal changes in the biochemical composition of dominant macroalgal species along the Egyptian Red Sea Shore. Biology. **12** (3), **2023**.
46. HIDAYAT N.S.M., MOHAMMAD-NOOR N., SUSANTI D., SAAD S., MUKAI Y. The effects of different pH and salinities on growth rate and carrageenan yield of *Gracilaria manilaensis*. Jurnal Teknologi. **77** (25), 1, **2015**.
47. DE GÓES H.G., REIS R.P. Temporal variation of the growth, carrageenan yield and quality of *Kappaphycus alvarezii* (Rhodophyta, Gigartinales) cultivated at Sepetiba bay, southeastern Brazilian coast. Journal of Applied Phycology. **24** (2), 173, **2012**.

48. TOI H.T., ANH N.T.N., NGAN P.T.T., NAM T.N.H., HAI T.N. Effects of stocking densities and seaweed types as shelters on the survival, growth, and productivity of juvenile mud crabs (*Scylla paramamosain*). Egyptian Journal of Aquatic Research. **2** (1), **2023**.
49. HEALY L.E., ZHU X., POJIĆ M., SULLIVAN C., TIWARI U., CURTIN J., TIWARI B.K. Biomolecules from macroalgae – Nutritional profile and bioactives for novel food product development. Biomolecules. **13** (2), **2023**.
50. GANESAN M., TRIVEDI N., GUPTA V., MADHAV S.V., REDDY C.R., LEVINE I.A. Seaweed resources of Kerala coast. Botanica Marina. **62** (5), 463, **2019**.
51. YANG Y.-F., FEI X.G., SONG J.M., HU H.Y., WANG G.-C., CHUNG I.K. Growth of *Gracilaria lemaneiformis* under different cultivation conditions and its effects on nutrient removal in Chinese coastal waters. Aquaculture. **254** (4), 248, **2006**.
52. ROLEDA M.Y., HURD C.L. Seaweed nutrient physiology: Application of concepts to aquaculture and bioremediation. Phycologia. **58** (5), 552, **2019**.
53. REJEKI S., ARIYATI R.W., WIDOWATI L.L., BOSMA R.H. The effect of three cultivation methods and two seedling types on growth, agar content and gel strength of *Gracilaria verrucosa*. Egyptian Journal of Aquatic Research. **44** (1), 65, **2018**.
54. WIKURENDRA E.A., SYAFIUDDIN A., NURIKA G., ELISANTI A.D. Water quality analysis of Pucang River, Sidoarjo Regency to control water pollution. Environmental Quality Management. **32** (1), 133, **2022**.
55. STEDT K., TRIGO J.P., STEINHAGEN S., NYLUND G.M., FORGHANI B., PAVIA H., UNDELAND I. Cultivation of seaweeds in food production process waters: Evaluation of growth and crude protein content. Algal Research. **63** (6), **2022**.
56. WANG Q., LAN L., LI H., GONG Q., GAO X. Effects of nitrogen source and concentration on the growth and biochemical composition of the red seaweed *Grateloupia turuturu* (Halymeniaceae, Rhodophyta). Sustainability (Switzerland), **15** (9), **2023**.
57. FADHLULLAH M., PRASETYATI S.B., PUDOLI I., LO C. Preliminary economic potential evaluation of seaweed *Gracilaria* sp. biomass waste as bioindustry feedstock through a biorefinery approach: A case study in Karawang, Indonesia. 3BIO: Journal of Biological Science, Technology and Management. **4** (1), 42, **2022**.
58. FERREIRA M., SALGADO J.M., FERNANDES H., PERES H., BELO I. Potential of red, green and brown seaweeds as substrates for solid state fermentation to increase their nutritional value and to produce enzymes. Foods. **11** (23), 3864, **2022**.
59. DUCOUSSO-DÉTREZ A., FONTAINE J., SAHRAOUI A.L.H., HIJRI M. Diversity of phosphate chemical forms in soils and their contributions on soil microbial community structure changes. Microorganisms. **10** (3), **2022**.
60. LIAW W.K. Chemical and biological studies of fishponds and reservoirs in Taiwan (Reprinted.). Chinese-America Joint Commission on Rural Reconstruction. **1969**.
61. MENDES M., FORTUNATO D., COTAS J., PACHECO D., MORAIS T., PEREIRA L. Agar content of estuarine seaweed *Gracilaria* using different cultivation methods. Applied Food Research. **2** (2), 100209, **2022**.
62. SARKAR S., REKHA P.N., BISWAS G., GHOSHAL T.K., AMBASANKAR K., BALASUBRAMANIAN C.P. Culture potential of the seaweed, *Gracilaria tenuistipitata* (Rhodophyta) in brackishwater tide fed pond system of Sundarban, India. Journal of Coastal Research. **86** (1), 258, **2019**.
63. RESZ M.A., ROMAN C., SENILA M., TÖRÖK A.I., KOVACS E. A Comprehensive approach to the chemistry, pollution impact, and risk assessment of drinking water sources in a former industrialized area of Romania. Water (Switzerland), **15** (6), **2023**.
64. DU T., BOGUSH A., EDWARDS P., STANLEY P., LOMBARDI A.T., CAMPOS L.C. Bioaccumulation of metals by algae from acid mine drainage: A case study of Frongoch Mine (UK). Environmental Science and Pollution Research. **29** (21), 32261, **2022**.
65. NIZAM N.U.M., HANAFIAH M.M., NOOR I.M., KARIM H.I.A. Efficiency of five selected aquatic plants in phytoremediation of aquaculture wastewater. Applied Sciences (Switzerland), **10** (8), **2020**.
66. MAKMUR M., MULYANINGRUM S.R.H. The performance of tissue cultured seed of *Gracilaria verrucosa* seaweed cultivated in brackishwater pond of Luwu Regency, South Sulawesi. Media Akuakultur. **13** (1), 1, **2018**.
67. CHEN B., ZOU D., MA Z., YU P., WU M. Effects of light intensity on the photosynthetic responses of *Sargassum fusiforme* seedlings to future CO_2 rising. Aquaculture Research. **50** (1), 116, **2019**.
68. ROSEMARY T., ARULKUMAR A., PARAMASIVAM S., MONDRAGON-PORTOCARRERO A., MIRANDA J.M. Biochemical, micronutrient and physicochemical properties of the dried red seaweeds *Gracilaria edulis* and *Gracilaria corticata*. Molecules. **24** (12), 1, **2019**.
69. SYAM A.P., SUARDI SYARIFUDDIN M. Analysis of growth and agar content of *Gracilaria* sp. with a different location in the coastal waters of Luwu Regency. Fisheries of Wallacea Journal, **1** (1), 24, **2020**.
70. ROSMIATI R., HARLINA H., SURYATI E., DAUD R., HERLINAH H. Growth performance and quality of tissue-cultured seaweed seed *Gracilaria verrucosa* cultured using broadcast method in brackishwater pond, Sinjai Regency. Jurnal Riset Akuakultur. **14** (3), 145, **2019**.
71. NSA. Dried Seaweed Export Available online: https://grenare.com/dried-seaweed-export-quality/, **2015**.
72. CIRIK Ş., ÇETIN Z., AK I., CIRIK S., GÖKSAN T. *Gracilaria verrucosa* (Hudson) Papenfuss and determination of chemical composition. Turkish Journal of Fisheries and Aquatic Sciences. **10** (4), 559, **2010**.
73. ROUT G.R., SAHOO S. Role of iron in plant growth and metabolism. Reviews in Agricultural Science. **3** (1), **2015**.
74. KONG Y., ZOU P., SONG L.-M., WANG Z., QI J.-Q., ZHU L., XU X.-Y. Effects of iron on the algae growth and microcystin synthesis: A review. Chinese Journal of Applied Ecology. **25** (5), **1533**.
75. BAZZANI E., LAURITANO C., SAGGIOMO M. Southern Ocean iron limitation of primary production between past knowledge and future projections. Journal of Marine Science and Engineering. **11** (2), **2023**.
76. HARISH V., ASLAM S., CHOUHAN S., PRATAP Y., LALOTRA S. Iron toxicity in plants: A review. International Journal of Environment and Climate Change. **13** (8), 1894, **2023**.
77. ASCH F., BECKER M., KPONGOR D.S. A quick and efficient screen for resistance to iron toxicity in lowland rice. Journal of Plant Nutrition and Soil Science. **168** (6), 764, **2005**.
78. SINAGA P.H., ELFIANI YUSUF R., NURHAYATI YUNITA R., UTAMI D.W., GIRSANG S.S. Resistance

of local rice progeny to ferrous iron toxicity between locations, seasons, and salt application in tidal lands. Agronomy Research. **21** (Spl1), 376, **2023**.

79. MARTINS A.P., JUNIOR O.N., COLEPICOLO P., YOKOYA N.S. Effects of nitrate and phosphate availabilities on growth, photosynthesis and pigment and protein contents in colour strains of *Hypnea musciformis* (Wulfen in Jacqu.) J.V. Lamour. (Gigartinales, Rhodophyta). Revista Brasileira de Farmacognosia. **21** (2), 340, **2011**.
80. PAN J., SHARIF R., XU X., CHEN X. Mechanisms of waterlogging tolerance in plants: Research progress and prospects. Frontiers in Plant Science. **11** (2), **2021**.
81. MAMEDE M., COTAS J., BAHCEVANDZIEV K., PEREIRA L. Seaweed polysaccharides in agriculture: A next step towards sustainability. Applied Sciences (Switzerland), **13** (11), **2023**.
82. MUSTAFA A., SYAH R., KAMARIAH K. Soil characteristics under different canopy types of mangrove vegetation and different soil depths as biological indicators for brackishwater ponds in Mamuju Regency West Sulawesi Province. Jurnal Riset Akuakultur. **6** (1), **2011**.
83. BANIK U., MOHIUDDIN M., WAHAB M.A., RAHMAN M.M., NAHIDUZZAMAN M., SARKER S., WONG L., ASADUZZAMAN M. Comparative performances of different farming systems and associated influence of ecological factors on *Gracilaria* sp. seaweed at the south-east coast of the Bay of Bengal, Bangladesh. Aquaculture. **574**, 739675, **2023**.
84. VAN GENUCHTEN C.M., PEÑA J., AMROSE S.E., GADGIL A.J. Structure of Fe(III) precipitates generated by the electrolytic dissolution of Fe(0) in the presence of groundwater ions. Geochimica et Cosmochimica Acta. **127**, 285, **2014**.
85. IFANSYAH H., YANI J.A. Soil pH and solubility of aluminum, iron, and phosphorus in Ultisols: The roles of humic acid. Journal of Tropical Soils. **18** (3), **2013**.
86. DARIAH A., SUTONO S., NURIDA N.L. The use of soil conditioners to increase agricultural land productivity. Jurnal Sumberdaya Lahan. **9** (2), 67, **2015**.
87. BUNSOM C., PRATHEP A. Effects of salinity, light intensity and sediment on growth, pigments, agar production and reproduction in *Gracilaria tenuistipitata* from Songkhla Lagoon in Thailand. Phycological Research. **60** (3), 169, **2012**.
88. ANTON A. Growth and content of seaweed agar (*Gracilaria* spp) at various salinity levels. Jurnal Airaha. **6** (2), 054, **2017**.
89. SYNYTSYA A., COPÍKOVÁ J., KIM W.J., PARK Y.I. Cell wall polysaccharides of marine algae. Springer Handbook of Marine Biotechnology. **543**, **2015**.
90. MEIRINAWATI H., WAHYUDI A.J. Seaweed as bioadsorbent for nitrogen and phosphorus removal. Journal of Environmental Science and Sustainable Development. **6** (1), 183, **2023**.
91. BURIYO A., KIVAISI A. Standing stock, agar yield and properties of *Gracilaria salicornia* harvested along the Tanzanian Coast. Western Indian Ocean Journal of Marine Science. **2** (2), 171, **2003**.
92. BUAPET P., HIRANPAN R., RITCHIE R.J., PRATHEP A. Effect of nutrient inputs on growth, chlorophyll, and tissue nutrient concentration of *Ulva reticulata* from a tropical habitat. Science Asia. **34** (2), 245, **2008**.
93. COULOMBIER N., NICOLAU E., LE DÉAN L., BARTHELEMY V., SCHREIBER N., BRUN P., LEBOUVIER N., JAUFFRAIS T. Effects of nitrogen availability on the antioxidant activity and carotenoid content of the microalgae *Nephroselmis* sp. Marine Drugs. **18** (453), 1, **2020**.
94. LI S.X., WANG Z.H., STEWART B.A. Responses of crop plants to ammonium and nitrate N. In Advances in Agronomy. **118**, 397, **2013**.
95. MUSTAFA A., HASNAWI H., ASAAD A.I.J., PAENA M. Characteristics, suitability and recommendations for management of land in acid sulfate soil-affected brackishwater ponds for tiger prawn (*Penaeus monodon*) culture in Luwu Regency, Indonesia. Journal of Coastal Conservation. **18** (6), 595, **2014**.
96. PERRYMAN S.E., LAPONG I., MUSTAFA A., SABANG R., RIMMER M.A. Potential of metal contamination to affect the food safety of seaweed (*Caulerpa* spp.) cultured in coastal ponds in Sulawesi, Indonesia. Aquaculture Reports. **5**, **2017**.
97. MUSTAFA A., ASAAD A.I.J., RIMMER M.A. Land characteristics and suitability for tilapia culture at different seasons in brackish water ponds of Bontoa Subdistrict, Maros Regency, Indonesia. IOP Conference Series: Earth and Environmental Science. **473** (1), **2020**.
98. MUSTAFA A., KAMARIAH K., RATNAWATI E. Soil quality and its implication for brackishwater pond soil management option in East Java Province, Indonesia. IOP Conference Series: Earth and Environmental Science. **860** (1), **2021**.