Sequential patterns of essential trace elements composition in
Gracilaria verrucosa and its generated products

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Abstract. Gracilaria widely known as a source of essential trace elements. However this red seaweeds also has great potential for being developed into commercial products. This study examined the sequential pattern of essential trace elements composition in fresh Gracilaria verrucosa and a selection of its generated products, namely extracted agar, Gracilaria salt and Gracilaria residue. The sample was collected from a brackish water pond, located in north part Semarang, Central Java. The collected sample was then dried under the sun, and subsequently processed into aforementioned generated products. The Gracilaria salt was obtained by soaking the sun dried Gracilaria overnight in fresh water overnight. The resulted salt solution was then boiled leaving crystal salt. Extracted agar was obtained with alkali agar extraction method. The rest of remaining material was considered as Gracilaria residue. The entire process was repeated 3 times. The composition of trace elements was examined using ICP-MS Spectrometry. Collected data was then analyzed by ANOVA single factor. Resulting sequential pattern of its essential trace elements composition was compared. A regular table salt was used as controls. Results from this study revealed that Gracilaria verrucosa and its all generated products all have similarly patterned the composition of essential trace elements, where Mn>Zn>Cu>Mo. Additionally this pattern is similar to different subspecies of Gracilaria from different location and and different season. However, Gracilaria salt has distinctly different pattern of sequential essential trace elements composition compared to table salt.

1. Introduction
Seaweeds are important marine resources that contains high amounts of vitamins, essential amino acids dietary fibers, minerals and essential trace elements. Gracilaria is seaweeds that flourish in brackish water shrimp ponds. This fast growing algae is edible and can be produced into commercial product such as antimicrobial substances and agar. The consumption of seaweeds is assumed to have health benefits [1]. The health benefit of seaweeds consumption is typically associated with high fibre content, vitamins, minerals and trace elements [2]. Living body contains a small amount of trace elements. In humans, deficiency of trace element are often encountered to such clinical condition [3]. Essential trace elements in the human body include zinc (Zn), copper (Cu), chromium (Cr), selenium (Se), manganese (Mn), molybdenum (Mo), cobalt (Co) and iodine (I).

Trace elements play a significant role in certain enzyme activities. However, an excess of trace elements can lead to poisoning. Seaweeds contain higher concentrations of minerals and trace elements than terrestrial vegetables [4]. As such they can be used as dietary sources of minerals and trace elements [5]. Among the difference classes of seaweeds, there are significant differences in trace element contents [6]. This is indicated that the presence of these elements is closely related to species specific biochemical composition. Additionally trace elements compositions also affected by
morphological and seasonal variation [7]. Subspecies of *Gracilaria* are important for industrial and biotechnological purposes and are considered economically valuable resources, because of their ability to produce high yields of commercially valuable biomass [8]. Finally, *Gracilaria* can be used as a multi products source for biotechnological, nutraceutical and pharmaceutical industries. In this study, we evaluate the sequential pattern of essential trace elements compositions in *Gracilaria* and its generated proceed product is evaluated.

2. Method
This study was conducted in Biology Laboratory, Diponegoro University and was conducted from June up until December 2018. *Gracilaria verrucosa* a red macroalga was collected from brackish water pond located in north Semarang, Central Java. The collected *Gracilaria* was washed in a brackish water to remove dirt. The cleaned *Gracilaria* was then brought to the laboratory and sundried to reach a stable water content. The dried sample was subsequently processed to get generated products, namely *Gracilaria* salt, extracted agar and residual compact waste. *Gracilaria* salt was produced by soaking the sample in twice its volume of water overnight. The resulting solution was then boiled until salt crystals formed. *Gracilaria* was treated with alkali solution, followed by boiling for approximately 30 minutes. Solution was filtrated, resulting in two products, namely extracted agar and its residual compact waste. The agar solution was frozen to get its gelatine form, followed by drying and milling to produce the agar powder. All processes was repeated 3 times. The generated products then had their trace element composition analysed using ICP-MS (Inductively Couple Plasma Mass Spectrometry). The resulting data was analized using ANOVA single factor, after which the sequential patterns of the essential trace elements compositions were compared. Table salt was used as control for sequential patterns in *Gracilaria* salt.

3. Results and Discussion
3.1. Essential Trace Elements in Fresh *Gracilaria verrucosa*
Seaweed has an important function as a mineral resources. In this study, laboratory analysis of eight trace elements in *Gracilaria verrucosa* showed that almost all essential trace elements are present in very small amount, manganese (Mn) being the exception. Manganese was present with approximately 1.42 ppm, whereas the other essential trace elements had quantities of less than 1 ppm. From these, Zn is present in highest amount (0.19 ppm), while other elements are present in amount of less than 0.1 ppm. These were copper (0.098 ppm), followed by molybdenum (0.052 ppm), chromium (0.027 ppm), selenium (0.007 ppm) and cobalt (0.002 ppm). When it is expressed as a sequence, the essential trace elements that are contained in *Gracilaria* are Mn>Zn>Cu>Mo>Cr>Se>Co, as is depicted in figure 1.

![Fresh Gracilaria](image)

**Figures 1.** Sequential essential trace element composition in Fresh *Gracilaria verrucosa*, (Mn>Zn>Cu>Mo).
According to existing research, a similar pattern of sequential composition is found in \textit{Gracilaria folifera} namely Mn>Zn>Cu>Cr>Co \cite{10}. Additionally, there is a similar pattern of essential trace elements compositions in \textit{Gracilaria prassa}, which is the same as \textit{Gracilaria folifera}, Mn>Zn>Cu>Cr>Co. The patterns of trace elements contain in \textit{Gracilaria} seems to be similar across subspecies \cite{11}.

It has been found that from 20 species of marine algae, the pattern of trace elements composition is also similar, Mn>Zn>Cu>Cr>Se>Co \cite{12}. However, it is worth noting that \textit{Porphyra umbilicalis} from three different locations, contains zinc consistently higher than manganese. This is significantly different compared to other species, where manganese content is consistently higher than zinc. A recent review by Bonanno et al. (2017), found a similar pattern in sequential compositions of essential trace elements among seagrass, a green and red macroalga, namely Mn>Zn>Cu>Cr \cite{13}. With the exception of brown algae, the presence of zinc is higher than manganese. In general, the sequential pattern of essential trace elements in marine plants is similar.

3.2. \textit{Trace Element Composition in Generated Gracilaria products}

This study also evaluated the composition of essential trace elements in generated products derived from \textit{Gracilaria}, namely, \textit{Gracilaria} salt, extracted agar and \textit{Gracilaria} residue.

3.2.1. \textit{Extracted Agar}. \textit{Gracilaria} is a seaweeds species that is used in several commercial products. The most significant product is agar. Among other products developed with this red alga are biosalt and its residue that can be further developed into soil conditioner or compost. This study examined the sequential composition of trace elements in biosalt and its residue. The results indicated that these pattern is similar to trace elements compositions in Fresh \textit{Gracilaria}.

Additionally, agar extracted agar from \textit{Gracilaria} also has similar pattern namely Mn>Zn>Cu>Mo. Studies on trace elements compositions in extracted agar is rarely done. Usually, trace elements compositions are studied on the entire of seaweeds body. The sequential pattern of essential trace elements in extracted agar is presented in figure 2.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure2.png}
\caption{Sequential patterns of essential trace elements composition in extracted agar from \textit{Gracilaria} verrucosa, Mn>Zn>Cu>Mo.}
\end{figure}

3.2.2. \textit{Gracilaria salt}. Gracilaria salt was extracted from salty \textit{Gracilaria} prior to it being processed into several products. In this study, table salt was used as control to primarily compare the sequential trace elements composition pattern, in \textit{Gracilaria} salt (figure 3). The control sample was obtained from environmental brackish water. Whereas the pattern of the sequential composition of trace elements in \textit{Gracilaria} salt is similar to that in fresh \textit{Gracilaria}, Mn>Zn>Cu>Mo. The pattern in table salt is distinctly different. The predominant element in \textit{Gracilaria} is manganese, whereas in table salt the
The predominant element is copper followed by zinc, manganese, molybdenum and chromium or Cu>Zn>Mn>Mo>Cr (figure 4). A study by Atkinson (1995) shows there tends to be a similar pattern of certain essential elements on a variety of salts. Results of this study show Gracilaria has a unique sequential pattern of essential trace elements composition. This pattern is also similar to other Gracilaria from different locations and seasons. This is indicated that live plants have specific biosynthesis of their chemical elements, and the pattern of their composition can differ from pattern found in their environmental habitat. The sequences found indicate that the predominant elements is copper, followed by zinc.

![Figure 3. Sequential pattern of essential trace elements composition in Gracilaria salt (Mn>Zn>Cu>Mo)](image)

3.2.3. *Gracilaria* residue. Residue from processed Gracilaria can be used to create products such as soil conditioner, paper and compost. All of these generated products carry a similar composition pattern of essential trace element, namely that of the pattern of essential trace elements compositions found in Gracilaria (figure 5).

![Figure 4. Sequential pattern of essential trace elements composition in table salt where Mn>Zn>Cu>Mo](image)
4. Conclusion
As an important source of trace elements, *Gracilaria* and its generated products have similar patterns of essential trace elements compositions, namely Mn>Zn>Cu>Mo. Some other species of seaweeds carry similar patterns, although there are slight differences. However, if compared to table salt, *Gracilaria* salt has significantly different sequential patterns of trace elements compositions.

Acknowledgement
The authors thank and acknowledge Diponegoro University for its financial support for making this study possible.

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