The use of Macroalga *Sargassum* sp. and *Gracilaria verrucosa* in improving Sandy and Clay Soil fertility

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Abstract. Macroalgae, such as *Sargassum* and *Gracilaria* have a great potential to be used as soil conditioner. Addition of macroalgae powder into a certain type of soil could significantly change soil fertility. The presence of gelatinous material in *Sargassum* and *Gracilaria* are predicted to be able in changing the chemical characteristic of soil, including organic material content, pH and C/N ratio. This study was focused on the evaluation of soil fertility sandy and clay soil after addition of *Sargassum* and *Gracilaria* powder as a soil conditioner. The collected macroalga was dried and soaked in fresh water several time to lower its salinity to reach into a normal level. The dried powder of *Sargassum* and *Gracilaria* were added and mixed through into sandy and clay soil (in a composition of 25%:75%). Treated soil was measured for their physical, chemical and physiological properties changes. Each treatment was replicated three times. Data were analyzed using ANOVA single factor and t-test. Results indicated that the addition of soil conditioner from macroalga *Sargassum* and *Gracilaria* were significantly increasing organic material content. Addition of *Sargassum* powder increased organic material more than *Gracilaria*. C/N ratio in sandy and clay soil was reduced by the presence of this both soil conditioner. Therefore, there was a strong indication in increasing in supporting for more soil fertility. The presence of macroalga was proven also in reducing pH to a normal level. It is recommended to develop this material to improve soil fertility and plant growth.

1. Introduction
Macroalga also knew as seaweeds has been widely known to have so many uses, such as fertilizer, soil conditioner, animal feed, cosmetic, biofuel, integrated aquaculture, waste treatment, etc. These marine plants have also already known as a rich natural resource for bioactive compounds [1,2]. Macroalgae, also known as seaweeds, produce many biologically active phytochemicals, which include among others, carotenoids, terpenoids, xanthophylls, chlorophylls, phycobilins, polyunsaturated fatty acids, polysaccharides, vitamins, sterols, tocopherol and phycocyanins [3].

Macroalgae (seaweeds) have been used by humankind for generations as a food and soil conditioning or fertilizer [4]. The current uses of seaweeds include human foods, fertilizers, phycocolloids and cosmetic ingredients [5], with Asia being the main market [6]. However, seaweed is still considered an under-utilized resource worldwide [7]. *Sargassum*, the rapidly growing macroalga has a naturally high content of antioxidants, carotenoids, and phenols, including the well-known anti-cancer compound fucoxanthin, making this species a potential source of a range of pharmaceutically relevant materials [4].

*Gracilaria Greville* genus (*Gracilariales*, Rhodophyta) is represented by more than 300 species of which 160 have been accepted taxonomically [8]. The macroalgae belonging to this genus are important for industrial and biotechnological uses and are considered economically valuable resources, because of their ability to achieve high yields of commercially valuable biomass [9].
Sargassum is a brown macroalga which is distributed throughout the temperate and tropical ocean of the world. They generally inhabit shallow water and coral reefs, and as free-floating species. It is commonly drifted and found on the beach.

In some region, Sargassum is collected for food resource and fertilizer. It is also being considered as medicinal sources [10]. Polysaccharides, terpenoids, phlorotannins, fucoids, sterols, and glycolipids obtained from marine macroalgae showed a wide range of pharmacological properties which includes anticancer, anti-inflammatory, antimicrobial, antiviral, antioxidant, hypoglycemic, hepatoprotective and neuroprotective activities [11, 12, 13].

Seaweeds have been developed into fertilizer products and have been a claim to make better germination, deeper root penetration, increase nutrient uptake and higher yield of the treated crop [14]. Seaweed has been used as fertilizer since the 19th century. However, it was applied only on limited to coastal areas [15]. Worldwide coastal communities have been traditionally using drifted seaweed as soil conditioner [16]. Eyras et al. [2013] had been evaluated the effect of seaweeds conditioner application on increase yield of tomato [17]. The use of seaweeds Gracilarie verrucosa and Sargassum sp. to increase the growth of Vigna radiata in sandy and clay soil also have been studied by Izzati [18]. This study is aimed to evaluate the effect of using these two seaweeds species on sandy and clay soil properties.

2. Research Methods
This study was conducted at Laboratory of Plant Physiology, Departement of Biology, Diponegoro University. The seaweeds, Gracilarie verrucosa and Sargassum sp. used in this study were collected from Jepara sea, Central Java. Collected seaweeds were washed thoroughly to remove contaminant dirt and let dry under the sun to get its minimum water content. Dried seaweeds were then soaked within fresh water to remove salt and reduced salinity. This work was done several time to get their salinity reaching zero (0 ppt). This was then followed by drying for the second time and continued by milling to get seaweeds powder. The dried seaweed powders were then used as a soil conditioner. As much as 10% of seaweeds powder was added into 40cm x 40cm x 40cm sized of polybag that already been filled with sandy and clay soil (approximately 90% of volume). The main media and soil conditioner were mixed thoroughly to get an even distribution. There were 4 treatments in this study. These were: a) 90% of sandy soil added 10% of Sargassum powder; b) 90% of sandy soil added by 10% of Gracilaria powder; c) 90% of clay soil added by 10% of Sargassum powder; d) 90% of clay soil added by 10% of Gracilaria powder; e) 100% sandy soil and 100% clay soil were used as controls. Each treatment were replicated 4 times. This experiment were designed by completed randomized design, using two factor. One factor is two type of soil (sandy and clay soil, as main media), whereas another factor is two type of soil conditioner (Sargassum and Gracilaria powder). Soil fertility parameters measurements include soil organic content, pH of the soil, ratio C/N, Water holding capacity and water infiltration of soil. Soil organic content was observed using dried combustion method. Soil pH meter was used to measured soil acidity. The ratio of C/N of soil was assessed by calculation of total carbon and total N in the soil. Water holding capacity was observed by measuring the volume of water retention by a certain volume of dried media. Water infiltration was reached by observing the period required for 100% of water volume to media volume to pass. Resulted data was collected and analyzed using ANOVA single factor to assume soil fertility differences.

3. Results and Discussion
3.1. Soil Organic Content
Organic content is one of important determinant for soil fertility. According to FAO (2017), soil organic matter is defined as a complex and continuous mixture of partially decomposed organic substances derived from plant litter [19]. It plays a crucial function and ecosystem is buffering against climate change. Hence, it supports food production and maintaining water availability. In this study, we evaluate the use of plant litter originated from seaweeds biomass from two species, Sargassum sp. and Gracilaria verrucosa. These two species have different potency in their biomass to support organic content in the soil. From this study, it resulted that, the addition of soil conditioner from seaweeds were significantly increased its organic content in both sandy and clay soil, as it is shown in Figure 1.
Figure 1. Organic content increase in media after addition of soil conditioner
From seaweeds. With Sargassum organic material in media was much higher compared to Gracilaria verrucosa in both sandy and clay soil.

From this study, it results that in controls (media without addition of any of this soil conditioner) indicated a significant difference in organic material content between sandy and clay soil. Clay soil has much higher organic material compared to sandy soil. Soil organic matter content is strongly related to soil texture [20]. According to Feller and Beare (1997), there is a strong relationship between soil texture and organic matter content. The more fine texture of the soil, the more organic matter content [21]. This is may because organic matter tends to more easily break down into smaller particle compared to soil particle itself. The higher content of organic matter will increase soil fertility. Soil organic matter is the most important determinant for the soil fertility.

Addition of soil conditioner will affect significantly on total organic matter in the soil. Addition of Sargassum powder can increase high organic matter than the addition of Gracilaria, both in sandy and clay soil. However, the different organic matter improvement between these two types of soil conditioner was not significant. From this parameter, it can be concluded that the addition of soil conditioner from seaweeds will significantly increase organic matter content in both, sandy and clay soil.

3.2. Soil pH
Soil pH is an indicator of soil acidity and has an important role in many chemical processes. It affects plant nutrient availability by regulating the chemical forms of nutrients. The optimum pH for most plants is ranged between 5.5 and 7.5 [22]. In this study, the addition of soil conditioner from seaweeds affects significantly on soil pH. Before this soil conditioner was applied, in control media, the pH of both media was around 8. After application of seaweeds as a soil conditioner, there was a strong indication of pH reduction close to normal, which is 7. This trend was performed by figure 2 as follows:
Figure 2. Effect of soil conditioner addition from seaweeds on soil pH. Both Sargassum and Gracilaria verrucosa were capable in reducing soil pH to normal (around 7).

The presence of soil conditioner from seaweeds was capable in changing soil pH from moderate alkaline to neutral. One of the important factors in determining soil acidity is decomposition of organic matter. Therefore, the more organic matter decomposition, soil pH will reduce more, as organic matter decomposition will produce more H+. In this study, it is found that the addition of seaweeds conditioner increases organic matter significantly. As a consequence decomposition of organic matter will increase, and the amount of H+ will also be increased. As a result, soil pH is reduced. It can be concluded that the addition of soil conditioner made from seaweeds will lower soil pH.

3.3. Soil C/N ratio

The C/N ratio is the mass of carbon compared to the mass of nitrogen in a particular substance. The C/N ratio is an important factor in determining soil fertility since it has a direct impact on residue decomposition and also nitrogen cycling in the soils. An optimum C/N ratio is 24. It is desired for decomposition crop residue. The higher ratio of C/N, the more carbon and the less Nitrogen content in the media. Moreover, C/N ratio less than 24 means the soil is more fertile as higher nitrogen content. Nitrogen is the most important nutrient to support plant growth as this mineral has a crucial function for protein synthesis and enzyme activity in metabolism. Addition of soil conditioner in certain media will affect a ration of C/N. As a soil conditioner particularly consists of plant litter will increase more carbon than nitrogen. In this study, it is found that in control media there is a significant different of C/N ratio between sandy and clay soil. Clay soil has a significantly higher ratio of C/N. The high amount of organic matter in clay soil is an important factor in determining high carbon content in this type of soil texture. The impact of soil conditioner addition on ratio C/N on sandy and clay soil is showed in the following figure 3:
Figure 3. Ratio of C/N performance on media added with soil conditioner made from seaweed Sargassum sp and Gracilaria verrucosa. The presence of soil conditioner will significantly change the ratio of C/N.

In sandy soil ratio of C/N is increase by the presence of soil conditioner from seaweeds. With Sargassum this increase is higher than addition of Gracilaira, his is indicated that addition of soil conditioner increase its organic carbon content, since in sandy soil the amount of this material is significantly lower. Contrary, the high organic carbon content in clay soil make the ratio of C/N is also very high. Addition of soil conditioner reduce this ratio of C/N significantly. It appear that this is caused by the increase amount of nitrogen in added soil conditioner. It has been widely known that seaweeds has high protein content. This is explain why addition of seaweeds for soil conditioner will provide more nitrogen. Therefore the ratio of C/N will be much lower. According to Fleurence (2004), the amount of protein in marine algae are vary according to species. Brown seaweeds content lower protein (3±15% of dry weight) compared to green (9± 26% of dry weight) and red seaweeds (reach to maximum level of 47% of dry weight) [23]. As red seaweeds, Gracilaria can support more protein and nitrogen availability in the soil, therefore the ratio of C/N is much lower then addition of Sargassum as brown seaweeds.

4. Conclusion
The chemical changes as soil fertility indicator on sandy and clay soil after addition of seaweed species Sargassum sp and Gracilaria verrucosa as soil conditioner has been evaluated. Results indicated that this two seaweeds species is capable in increasing soil fertility. Addition of seaweeds soil conditioner can improve soil organic content, change pH into normal level and reduce ratio of C/N in both, sandy and clay soil. Gracilaria give a better results in lowering C/N ratio compared to Sargassum, as this red seaweeds is usually has higher content of protein than brow seaweeds.

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