Analyses of organic matter and heavy metal composition in formulated macroalgae-based organic fertilizer

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Abstract. The application of inorganic fertilizers has been continuously increasing since last many decades globally. Farmers nowadays heavily rely on the use of inorganic fertilizers to meet the production of their farms and gardens. This becomes a concerning environmentally and also health issue. One solution is to decrease the use of inorganic fertilizers and substitute with organic fertilizers. In our previous work, we have developed macroalgae-based organic fertilizer (MbOF) which show promising results in increasing the growth and yield of various crops and plants. However, the quality of the formulated MbOF has never been accessed. In this study, we analysed the organic matter composition and heavy metal safety profile of the MbOF. Our results show that MbOF contains considerably high organic C (43.05 ± 2.56 %). The cumulative content of total-N (1.91 ± 0.78 %), P2O5 (2.20 ± 0.81 %) and K2O (2.18 ± 0.54 %) were also above 4%. Furthermore, the C/N ratio was sufficient (22.54 ± 5.81 %). In addition, heavy metal analyses also show low content of Pb (5.61 ± 0.71 ppm) and Hg (0.29 ± 0.14 ppm). Based on the results, the formulated MbOF could be employed as substitute or used together with inorganic fertilizer to get maximum benefits regarding yield and growth of crops and plants.

1. Introduction

The rapid growth of human population leads to the high demands of food production [1]. Hence, this results in the intensive agriculture which leads to excessive use of fertilizers. However, the common fertilizers used in nowadays agriculture practice is commonly chemical or more known as inorganic fertilizers. Inorganic fertilizers usually are most nitrogenous fertilizers. Although nitrogenous-based fertilizers contribute to substantially increasing plant yield [2]. But, along with this there are also negative effects to the environment and also human health. Hence, currently potential natural resources are investigatd to be used as a substitute for inorganic fertilizers. Macroalgae or seaweeds which refer to the wide group of macroscopic multicellular marine algae has been used as manufactured extracts in various industries including agriculture [3,4]. Several beneficial effects have been demonstrated for crops and plants grown with macroalgae or macroalgae extracts. Our
previous research also demonstrated that macroalgae application could increase growth and yield in rice plants and tomato [5–7]. The current formulated macroalgae-based organic fertilizer (MbOF) is composed of three dried brown seaweeds Sargassum cristaefolium, Sargassum crassifolium, and Sargassum polycystum. Brown seaweeds are known for their fast growth, which suggests the presence of growth phytohormones. In addition, previous studies have detected valuable phytohormones in brown seaweeds which could be applied as biofertilizers.

Current work focuses on the quality assessment of MbOF regarding the organic matter composition such as organic C, P2O5, K2O, and C/N ratio. In addition, as macroalgae is also known as an efficient absorbent [8]. Heavy metal content is also required.

2. Materials and methods
The values obtained were compared with the minimum requirements established by The Indonesian Ministry of Agriculture (no.70/Permentan/SR.140/10/2011) for solid organic fertilizer (Table 1).

Table 1. Certified requirements for solid organic fertilizer [9].

| Parameter       | Unit | Standard      |
|-----------------|------|---------------|
| Organic C       | %    | Min 15        |
| N + P2O5 + K    | %    | 4             |
| C/N ratio       |      | 15-25         |
| Pb              | ppm  | max 50        |
| Hg              | ppm  | max 1         |
| As              | ppm  | max 10        |

2.1. Macroalgae-based organic fertilizer (MbOF)
The macroalgae-based organic fertilizer (MbOF) was formulated based on three brown seaweeds which are Sargassum cristaefolium, Sargassum crassifolium, and Sargassum polycystum. The mixture of seaweeds was fermented with addition of EM4. The fermentation process was ended in 30 days, and the resulting biomass was used for further studies.

2.2. Determination of organic matter

2.2.1. Determination of organic C and total N
The organic carbon was determined by the procedure described by Walkley and Black using the dichromate wet oxidation method [10]. Total N was determined by the Kjeldahl digestion method [11].

2.2.2. Determination of P2O5

Phosphorus (P2O5) is an essential macro nutrient that is very important for plant growth. Phosphorus measurements can be carried out using the spectrophotometric method. Standard solution of parent P2O5 with concentration of 0; 100; 200; 300; 400; 500; 600; 700; 800; 900 and 1000 mg/L pipetted as much as 5 mL into a 100 mL measuring flask yielding a concentration of 0; 5; 10; 15; 20; 25; 30; 35; 40; 45 and 50 mg/L. Standard parent solution and blank (without standard parent solution) added 5 mL of ammonium molybdate vanadate (1:1) reagent and then homogenized with distilled water to stand for 30 minutes were measured using UV-Visible spectrophotometer at \( \lambda = 420 \) nm [12].

2.2.3. Determination of K2O

10 g of soil sample was dissolved in 100 mL of 0.01 M NH4OAc at pH 7.0 for 1 hour, then the cations were measured in the supernatant using an Atomic Absorption Spectrometer (AAS). The potency of K2O extracted using 25% HCl was then measured using AAS for K. The available macro and
micronutrients were extracted with a mixture of sodium acetate and DTPA using a modified Morgan Wolf method [13].

2.3. Determination of heavy metal contents

2.3.1. Determination of Hg content
Hg was analyzed using a cold-vapor Atomic Absorption Spectrometer (PerkinElmer Flow Injection Mercury Systems 400, PerkinElmer; Wellesley, MA, USA). 500 µL of the soil sample solution was prepared in a mixture of 1.1% (v/v) SnCl2 in 3% (v/v) HCl. Standard reference materials were included in each batch run to ensure analytical quality [14].

2.3.2. Determination of Pb content
1 g of dry soil sample was put in a 250 mL beaker separately with 15 mL of aquaregia (35% HCl and 70% high purity HNO₃, in a ratio of 3:1). The mixture was then dissolved at 70°C until the solution became transparent. The resulting solution was filtered through whatman filter paper no. 42 and into 50 mL dilute to 50 mL volumetric flask and diluted to volume mark using deionized water and the sample solution was analyzed for Pb concentration using an atomic absorption spectrophotometer [15].

2.3.3. Determination of As content
A soil of 1 g was placed in a 50 mL beaker and extracted four times with a 5 mL portion of concentrated HCl. The mixture was boiled for about 30 min. The solution was cooled and diluted in 25 mL of distilled water. Aliquotes of the sample were analysed by the reported method [16].

3. Results and Discussion

3.1. Organic matter composition of MbOF

The organic C content obtained was 43.05 ± 2.56 %. Carbon source is essential for plant optimum growth [17]. Furthermore, this value is also higher compared to other studies of organic fertilizer from manures such as rabbit (30.1 %), cow dung (26.5 %), and pig manure (20.1%). The N (1.91 ± 0.78 %), P (2.20 ± 0.81 %), K (2.18 ± 0.54 %) composition was also sufficient (Figure 1). In addition, the total N, P, K value was higher compared to the certified 4% (Figure 1B). The total N content was lower compared to organic manure such as poultry manure (4.87 %), cow manure (2.39 %) and compost (2.16 %). However, the P₂O₅ content is almost the same as obtained in previous study [18]. In addition, the K₂O content is actually higher compared to organic manure reported in previous studies [19]. The potassium content in seaweeds has also been reported to be significantly high in Indonesian brown seaweeds such as Sargassum [20]. Potassium is an essential nutrient required by plants that effects most of the biochemical and physiological processes that induce growth. In particular, the plants ability to survive in various biotic and abiotic stresses [21].
The Nitrogen (N) content is essential for the growth of plants. It could be present in two ways in organic fertilizers, in mineral form and organically bound. Hence, to meet the required N needs for plants, optimal concentration of MbOF could be applied with precise concentration of inorganic fertilizer to optimize growth and yield of plants as shown in previous studies [5]. Furthermore, the N cycle could actually be improved by addition of seaweed fertilizer by improving N fixating microbiota profiles in the soil [22]. Another study also reported to increase N content in seaweed-based fertilizer by addition of fish waste compost [23].

3.2. Heavy metal analyses in MbOF

The marine ecosystem including Indonesia is severely affected by contamination of heavy metals. Heavy metals mainly Hg and Pb holds a critical role in the marine environment [24]. Because even at low concentrations, they pose a severe risk to the environment and also human health. Previous reports have stated the ability of macroalgae to absorb heavy metals [25]. As in some cases, macroalgae are used as a bioindicator for heavy metal contamination [26]. Figure 2 shows the Hg and Pb content in MbOF compared to the requirements stated by The Indonesian Ministry of Agriculture for solid organic fertilizers.
The heavy metals Hg and Pb along with As has shown several toxicity effects in the kidney and nervous system [27]. Hence, in this study these compounds were selected for analyses. However, there are also possibility of other heavy metal contaminants present in MbOF. Heavy metals such as Sn, As, and Ni are also commonly present in macroalgae in moderate to high concentrations [28]. Nevertheless, MbOF shows minimum amount of Hg, Pb, and As compared to the certified amount (Figure 2). It has been reported that plants are able to uptake this compounds and accumulate in their tissues [29].

4. Conclusion
In conclusion, our results show that the formulated MbOF contains sufficient organic matter in regards to N, P, and K content. Hence, MbOF could be potentially used together with inorganic fertilizer with the appropriate concentrations to obtain maximum benefits such as growth and yield. In addition, the combination of MbOF with inorganic fertilizers in agricultural systems could decrease environmental contamination due to excessive use of nitrogen-based fertilizers.

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