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Phytoremediation of Maninjau Lake water using Minute Duckweed (*Lemna perpusilla* Torr.)

T Chrismadha¹, T Suryono², M Magfiroh¹, Y Mardiaty¹, and E Mulyana¹

¹Research Center for Limnology, Indonesian Institute of Sciences, CSC-BG Cibinong, Jl. Raya Bogor Km 46, Cibinong 16911, Indonesia

²Unit for Technology Transfer of Lake Restoration – Indonesian Institute of Sciences (UTTTLR-LIPI), Jalan Raya Lubuk Basung Km. 4 Kabupaten Agam 26471, Sumatra Barat, Indonesia

Email: tjandra@limnologi.lipi.go.id

Abstract. Lake Maninjau is a tecto-volcanic lake in West Sumatra, which is currently facing eutrophication problem and urgently needs measures to be recovered. A preliminary experiment was carried out to assess the possibility of using minute duckweed (*Lemna perpusilla* Torr.) for healing the lake water, while also producing biomass for alternative feed. This experiment was carried out in The Unit for Technology Transfer of Lake Restoration – Indonesian Institute of Sciences, which located next to Maninjau Lake. It employed 9 plastic containers of 30X20X20 cm³ filled with lake water taken from 3 locations of different level nutrient contamination. As much as 10 g minute duckweed was put into each container and let to grow for 9 days. Observation on the plant growth and biomass productivity along with the water nutrient concentration was carried out every 3 days. At the end of experiment duckweed biomass was sampled for proximate analysis. The results shows that Maninjau Lake water supported only sub-optimal growth of the duckweed, indicated by low growth rate (ranged 8-22 %/day) as well as the biomass protein content (13-15 % DW). The duckweed, however, can eliminate up to 94% phosphate and 54% total in-organic nitrogen from the water, which also indicating that phosphorous was the limiting factor for duckweed growth in the lake.

1. Introduction

Anthropogenic activities have been widely known to give severe impact on aquatic environment, as water bodies particularly rivers and lakes largely become dumping site of liquid wastes, both from domestics, industries, and husbandries. Figure of water damage due to human activities has been brought about by Garono (2001) whose reported contaminant loading in Citarum River, which flow along through dense populated area in Bandung, West Java as much as 338.92 ton BOD/day and 545.11 ton COD/day, while the nutrient input into Saguling Reservoir that located at the most high part of the river was calculated as much as 34.032 tons N/day and 5.5 ton P/day [1]. At the same time, Garono (2002) has also calculated contamination from floating cage fishery aquaculture in the reservoir were 10,952 ton organic/year, 478 ton N/year, and 68 ton P/year [2]. Those all together lead to water eutrophication, in which the reservoir water has been reported to contain 0.684 – 3.460 mg/L TN and 0.067 – 0.364 mg/L TP, while the chlorophyll content was as high as 5.364 – 71.126 mg/m³ [3][4].

A similar eutrophication problem is currently occurred in Maninjau Lake, a tecto-volcanic lake of 9737.50 Ha wide located in Agam, West Sumatra [5]. The problem mostly attributed to massive floating cage fisheries application in the lake [5-11]. Syandri *et al* (2014) has classified the lake water



status, particularly in the littoral zone being hyper-eutrophic, with total phosphorus content ranged 0.55-0.65 mg/L and chlorophyll-a ranged 236.03 - 297.01 µg/l [5]. Lukman (2013) reported seasonal and temporal dynamic of total phosphorous and total nitrogen content in the lake water which ranged 0.027 - 0.129 mg/L and 0.366 - 7.429 mg/L, respectively [12]. It is consistent with Sulastrri *et al* (2015) that reported a dynamic of phytoplankton density during 7 year observation of 2005-2011, which attributed to seasonal nutrient availability in the lake [7]. It is also pointed out that nutrient contamination has led to blue-green algal bloom and severe the water quality so as unsuitable for swimming or bathing and further obstacles tourism development in the area.

Efforts for recovering the lake water quality, therefore, is badly required and indeed has become awareness of all the stake holders. A measure to reduce load of contamination, particularly by reducing floating cage fishery activities has been declared, but the implementation in the field has been hindered by lack of better alternative environmentally sound activities for earning life. At the same time, efforts for employing some remedial technologies has also been faced obstacle of economical means as they needs high investment as well as maintenance and operational cost. A better approach for resolving lake water quality problem is still have to find out, since the problem is naturally complex, particularly in terms of the anthropological aspect.

There is a new scientific approach to enhance environmental problem resolution by using plants called phytotechnology [13], which has been promoted including for water contamination problem resolution. It is actually based on the general concept that plants through the photosynthetic capability adsorb nutrients and converts it into biomass which can be used for any purposes [14]. Wide variety of plants enables a selection for any kind of superior capability plants in terms of water remedial function as well as for any specific uses of the generated biomass. In this case, selection can be directed to find out a plant that has ability to carry out integrative multiple purposes, such as water quality healing and feed.

Previous study in RC Limnologi – Indonesian Institute of Sciences has revealed that use of minute duckweed (*Lemna perpusilla* Torr.) could handle water quality of a closed water recirculation pond while producing biomass for additional feed, which increase the fish production eiffecientcy as much as 20-30% [15]. Group of duckweeds have indeed been widely reported to be useful for phytoremediation of water contamination [15-21]. For example, *Lemna minor* have been reported to have an ability to adsorb NH₄ and NO₃ [22]. Under in vitro condition, the adsorption rate on nitrogenous and phosphorus compounds was as high as 3.36 g/m²/day and 0.20 g/m²/day, while in the field the absorption rate was 2.11 g/m²/day and 0.59 g/m²/day, respectively [16]. El-Kheir *et al.*, (2007) have also reported the ability of *Lemna gibba* to eliminate various parameters, including TSS, BOD, COD, NO₃, NH₄, o-PO₄, Cu, Pb, Zn, and Cd by as much as 96.3%, 90.6%, 89.0%, 100%, 82.0%, 64.4%, 100%, 93.6%, and 66.7%, respectively [17]. At the same time, Alaert *et al.* (1996) reported that duckweed removed 74% TKN and 77% TP in waste water treatment ponds at 21 days retention time, resulted in an acceptable low nutrient water output of 2,7 mg/L TKN and 0,4 mg/L TP [23]. Chrismadha and Mardiaty (2012) reported that *L. perpusilla* was able to grow on Saguling Reservoir eutrophic water, and eliminated the N-NO₃ and P-PO₄ in the water up to 75% in 3 days [21].

There has also been some reports pointed out a great potential of duckweed to be an alternative feed for fishes, duck, cow, and pig [24-27]. It is based on the facts that some duckweed have high protein content of 10-43% DW, as well as the amino acids composition that closes to animal protein so as more suitable to be used for feed [24]. Furthermore, under optimal growth condition the plant can be the sources of various minerals, such as K and P, and pigments, particularly carotenes and xanthophyll's.

Beside those nutrition benefits, the plants belong to family of lemnaceae also known to have advantages in terms of high biomass productivity. Landesman *et al.* (2005) reported that the biomass doubling time of duckweed under optimal condition was only 2 days [25], and under an effective culture system could achieve 13-38 tons DW/Ha/year [18]. Landesman *et al.* (2005) also pointed out the duckweed high protein productivity which can achieve up to 6-10 times higher than that of soy bean cultivation [25].

One of duckweed live in area around Maninjau Lake is *L. perpusilla* Torr. which known under local name 'mata lele', which found massively growth particularly in paddy field. This species was then considered to be employed for taking advantage of eutrophic lake water to produce biomass that can be promoted for additional feed for tilapia culture around there. Nutrient elimination from the water can be expected occurred along with the plant growth and harvesting, so that also can take a part in effort to enhance the lake water quality. This study carried out a preliminary trial to grow the plant based on Maninjau Lake water media, with evaluation focused on the growth ability and nutrition status as well as the nutrient elimination capacity.

2. Materials and Methods

2.1. Source of duckweed *L. perpusilla* Torr.

The experimental minute duckweed used was originated from those naturally grown from area surrounding the lake, particularly paddy fields. The plant was collected and stocked in ponds at Unit for Technology Transfer of Lake Restoration – Indonesian Institute of Sciences (UTTTLR-LIPI) located next to Maninjau Lake, with water source came from adjacent paddy field and fish ponds. The plant lives consuming nutrients released from the fish metabolisms as well as from the excessive feed.

2.2. Source of lake water

Lake water used in this experiment was taken from 3 different sites, namely: Muko-muko, Maninjau, and Sungai Batang. Selection of those 3 sites was representing variation in lake water status in terms of nutrient contamination. The lake water was pumped up to polycarbonat containers, transported to UTTTLR-LIPI and stored also in polycarbonate containers before use. Physical parameters (pH, DO, conductivity, TDS) measurement as well as water sampling for total inorganic nitrogen (TIN) and P-PO4 (TP) analysis were performed prior the experiment (Table 1).

2.3. Growth and contaminant elimination experiment

The experiment was carried out using 9 plastic containers (30X20X20 cm³), placed in a partly transparent roofed area, where there was direct illumination of morning sun light while shaded in the rest of the day. However, there were mostly cloudy days during experimental time. The lake water was filled into the experimental containers made up 15 cm depth with 3 replicates for each site water. As much as 5 g minute duckweed was stocked into each container and let to grow making do the fertile lake water for 9 days (18 – 27 April 2018), in which the biomass development were observed every 3 days, by taking up all the biomass from the container for weighing and put it back to the container after then. Biomass samples were taken at final observation and sent to RC Biotic Resources and Biotechnology – Institute of Research and Community Development – Bogor Agriculture University Laboratory for proximate analysis according to [28][29]. Water samples for TSS, TIN and TP analysis, as well as water quality (temperature, pH, DO, conductivity, TDS) were also taken every 3 days. TSS was analyzed gravimetrically. TIN data was obtained by determining N-NH₄, N-NO₂, and N-NO₃ concentration in the water and sum them up together. Since the turbidity was so high that might interferes spectrophotometry, filtered water was subsequently used for N-NH₄, N-NO₂, N-NO₃ and TP analysis, which were performed following the methods of [30]. For water quality meter were pH meter (Lutron pH-201), conductivity and TDS-meter (Lutron YK-2001PH), and DO-meter (Lutron DO-201).

2.4. Data analysis

Plant growth and productivity was evaluated according to the observed fresh biomass weight obtained from regular observation. Plant specific growth rate (SGR) was calculated according to the equation as follow:

$$SGR = \frac{\ln X_t/X_0}{t} \times 100 \quad (1)$$

Table 1. Initial water quality Maninjau lake water used in the experiment

Parameters	Units	Values		
		Muko-muko	Maninjau	Sungai Batang
Temperature	°C	24.9 – 25.1	25.1 – 25.8	25.3 – 25.8
pH		8.70 – 9.34	8.75 – 9.32	8.95 – 9.33
DO	mg/L	5.4 – 6.1	5.6 – 5.9	5.2 – 5.6
Conductivity	mS/cm	0.111 – 0.113	0.112 – 0.113	0.110 – 0.113
TDS	mg/L	75	75	73 - 75
Turbidity	NTU	10.01 – 16.89	8.83 – 11.65	9.87 – 13.54
TSS	mg/L	19.5	19.5	13.5
TIN	mg/L	0.123 – 0.182	0.119 – 0.144	0.126 – 0.141
TP	mg/L	0.057 – 0.065	0.050 – 0.055	0.055 – 0.065

in which SGR = plant specific growth rate, X_t = plant biomass at time t , X_0 = plant initial biomass, and t = time (day). While plant productivity was calculated according to a formula as follow:

$$P = \frac{W_t - W_0}{t \cdot A} \quad (2)$$

in which P = plant productivity, W_t = plant biomass at time t , W_0 = plant initial biomass, t = time (day), and A = pond area.

Duckweed capacity to control nutrient contamination is expressed by means of nutrient elimination rate, involving parameters of TN and TP. The calculation was based on the nutrient concentration decreasing pattern in the media water that occurred along with the plant biomass growth, which then extrapolated to the nutrient elimination capacity according to a formula, as follows:

$$ER = \frac{C_0 - C_t}{C_0} \times 100 \quad (3)$$

in which: ER = nutrient elimination rate of the duckweed (%), C_0 = initial nutrient concentration, C_t = nutrient concentration at time t .

3. Results and Discussion

3.1. Maninjau lake water

Maninjau lake water condition after sampling at 3 locations were shown in Table 1, while dynamic of the water during experimentation time was presented in Table 2. The lake is naturally located in relatively high altitude that give a consequence of low temperature of 24 - 26 °C. Even though Leng (1999) [24] has stated that group of duckweed grow in temperature range of 6 - 33 °C, but in general each species of duckweed has its own optimal growth temperature [31]. Minute duckweed used in this experiment is *L. perpusilla* Torr. that has natural habitat world wide in low and medium altitude of tropical region [32], so as although there is plenty of sporadic population bloom, mainly in paddy field

surrounding the lake, but the growth is possibly not as fast as that in the lower land. It is supported by Park *et al* (2013) [31] that showed optimal growth temperatur for 3 species of minute duckweeds (*Lemna gibba*, *Lemna minor*, and *Lemna paucicostata*) were at 30 - 35 °C.

Tabel 2. Water quality dynamics during experimental time

Sites	Day-0	Day -3	Day -6	Day-9
Temperature (°C)				
Muko-muko	25.07 ± 0.15	24.93 ± 0.21	23.20 ± 0.10	23.03 ± 0.72
Maninjau	25.40 ± 0.36	24.90 ± 0.30	23.13 ± 0.15	23.33 ± 0.35
Sungai Batang	25.37 ± 0.40	24.97 ± 0.21	23.23 ± 0.23	23.33 ± 0,25
pH				
Muko-muko	9.12 ± 0.37	9.39 ± 0.05	8.26 ± 0.27	7.78 ± 0.22
Maninjau	9.12 ± 0.32	9,40 ± 0.07	8.36 ± 0.40	7.80 ± 0.34
Sungai Batang	9.20 ± 0.22	9.41 ± 0.06	8.36 ± 0.31	7.753 ± 0.20
DO (ppm)				
Muko-muko	5.74 ± 0.35	5.00 ± 0.26	4.23 ± 0.38	4.67 ± 0.06
Maninjau	5.73 ± 0.15	5,07 ± 0.12	4.33 ± 0.21	4.57 ± 0.31
Sungai Batang	5.40 ± 0.20	5.17 ± 0.25	4.30 ± 0.10	4.60 ± 0.00
Conductivity (mS/cm)				
Muko-muko	0.11 ± 0.00	0.11 ± 0.00	0.11 ± 0.00	0.11 ± 0.00
Maninjau	0.11 ± 0.00	0.11 ± 0.00	0.11 ± 0.00	0.11 ± 0.01
Sungai Batang	0.11 ± 0.00	0.11 ± 0.00	0.11 ± 0.01	0.11 ± 0.01
TDS (ppm)				
Muko-muko	75.00 ± 0.00	74.67 ± 0.58	77.00 ± 1.00	71.67 ± 3.06
Maninjau	75.00 ± 0.01	75.00 ± 0.02	75.00 ± 2.65	72.67 ± 6.66
Sungai Batang	74.33 ± 1.15	75.33 ± 0.58	72.67 ± 4.73	72.00 ± 5.66
Turbidity (NTU)				
Muko-muko	12.80 ± 3.62	7.78 ± 1.58	8.11 ± 1.66	9.97 ± 2.56
Maninjau	11.65 ± 2.93	7.77 ± 1.52	9.74 ± 1.76	9.31 ± 2.31
Sungai Batang	11.70 ± 1.84	7.21 ± 1.37	8.40 ± 1.29	6.74 ± 3.37

Measurement of pH value showed that the lake water was tend to be alkaline with pH value 8.70 - 9.34, in which a gradual declination was observed during 9 days experimentation time down to 7.75 - 7.78. This phenomenon can be associated with trophic status at the sampling time, in which fertile water vaped phytoplankton bloom resulting in intensive photosynthetic process that absorb CO₂ from water and give consequence of pH inclination. Meanwhile, in the experimental containers nutrient was consumed by duckweed which although also performed intensive photosynthesis but taking CO₂ from the air, so as less CO₂ uptake from the water while diffusion process from air slowly

increase the water CO₂ concentration and lower the pH. This range of pH is still under tolerance value for minute duckweed growth [24].

DO concentration of > 5 mg/L is considerably high to support healthy living in the water. Even though slightly decrease during experimentation was occurred, but the value still > 4 mg/L, and beyond the lower limit of requirement for aquatic life [33]. In contrast, electrical conductivity parameter showed value of 0.111 - 0.113 mS/cm which was remarkably lower than that expected for a eutrophic water. A higher conductivity value of around 1.13 - 1.14 mS/cm has been reported by Sulastri *et al* (2016) [8] in April 2014. This range of conductivity value was also markedly lower than that reported for hypereutrophic water of Lake Limboto which was ranged 0.209 - 0.340 mS/cm [34]. Electrical conductivity is a parameter to express ionic concentration in the water and can be associated with nutrient availability [35]. Wendeou *et al* (2013) [36] reported optimal conductivity for growth of greater duckweed (*Spirodella*) was between 0.6 - 1.0 mS/cm, and it points out possibly nutrient limitation to support minute duckweed growth in Maninjau lake water.

Turbidity and TSS values of Maninjau lake water were 8.83 - 16.89 NTU and 13.5 - 19.5 mg/L, respectively. This turbidity value was relatively low compared to other eutrophic waters, such as small lakes in West Java (Indonesia) those had turbidity values between 5.29 - 31.6 NTU [37] and Limboto Lake in Gorontalo of 15 - 222 NTU mg/L [34]. In contrast, the TSS value was remarkably higher than that in Lake Ranau in South Sumatra which was reported to be ranged 0.65 - 6.05 mg/L [38]. Both turbidity and TSS might be associated with water organic content and indicates a potential source of nutrients in the water. Lower turbidity and TSS was observed along with growth of minute duckweed during experimental time.

Maninjau lake water taken for this experiment contained TIN and TP ranged 0.119 - 0.183 mg/L and 0.050 - 0.065 mg/L, respectively. A slight higher nitrogen and phosphate concentration was observed in Muko-muko water compared to those in Maninjau and Sungai Batang. Syandri *et al* (2014) [5] has reported higher NP content of Muko-muko littoral water which was 0.55 mg/L TP and 1.28 mg/L TN, which was comparable with those concentration in Saguling River of West Java (TN 1.289 mg/L and TP 0.047 mg/L) [21]. This difference might be attributed to seasonal variation, such as that has been reported by Lukman *et al* (2013) [12], in which annual oscillation of TN in Maninjau lake water were between 0.336 - 7.429 mg/L and TP was 0.020 - 0.134 mg/L, respectively.

3.2. Duckweed growth and productivity

Minute duckweed was observed to grow sub-optimally in the Maninjau Lake water. The growth rate was relatively low, ranged 18-23 %/day, even in the early growth stage under ambient water nutrient concentration (Figure 1). Although it is slightly higher than that reported by Chrismadha *et al* (2014) where the plant average specific growth rate was ranged 11,95 - 17,63 %/day under sub-optimal condition of a closed water recirculation system of common carp fish pond[15], this value was markedly lower than those grew in a closed water recirculation system for cultivation of cat fish (*Clarias* sp.) which was in the range of 19 - 38 %/day [39][40]. This sub-optimal growth condition was also indicated by the biochemical composition, particularly that of low protein content (13-15 % DW) (Figure 2). This is comparable with Yilmaz *et al.* (2004) whose reported that *L. minor* biomass contained 18.38% protein [41], but these result is remarkably lower from those grown under vaporable conditions, such as that reported as much as 38.10 % DW [40] and up to 45 % DW [24]. Leng *et al.* (1995) have reported the influence of nitrogen to stimulate protein synthesis in greater duckweed (*Spirodella* sp.) [42], while under poor condition the plant accumulate more fibre. Leng (1999) has also pointed out the important of nitrogenous availability to support protein synthesis by minute duckweed [24]. Beside those, sub-optimal growth of minute duckweed was also indicated by long root morphology, as that has been reported by Chau (1998) that there is a negative correlation between root length and duckweed growth and protein content [43]. Minute duckweed grew in Maninjau lake water had longer root than those grown in fishery ponds. As mentioned above, this sub-optimal growth of minute duckweed in Maninjau can be attributed to nutrient limitation and sub-optimal temperature over there.

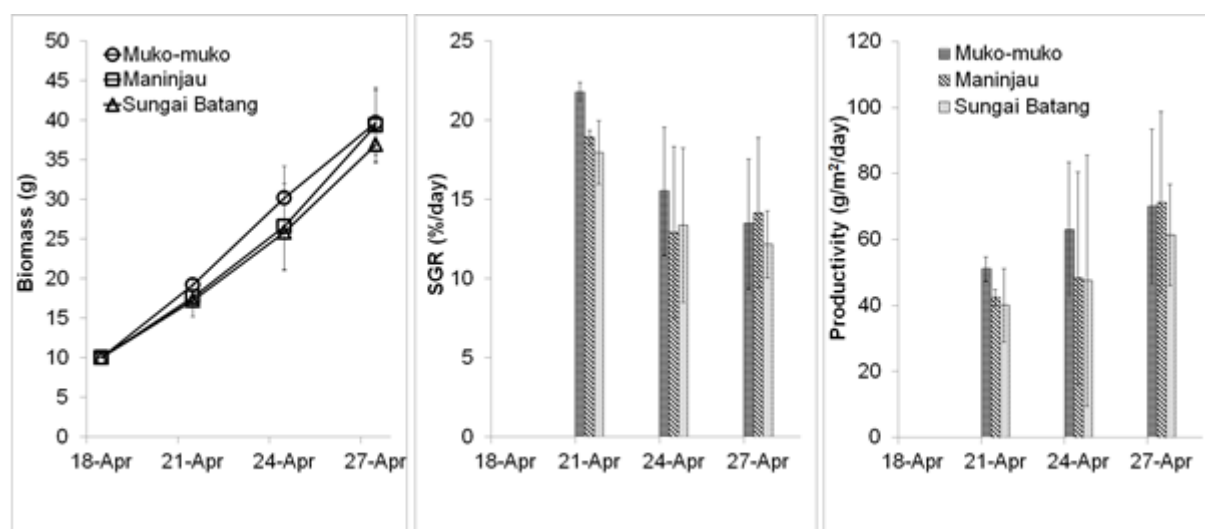


Figure 1. Growth and biomass productivity of minute duckweed grown in Maninjau Lake water

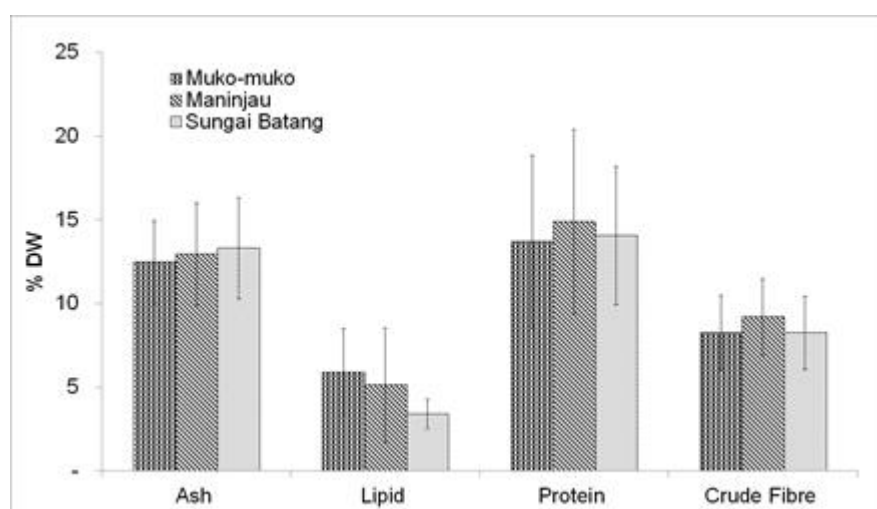


Figure 2. Proximate composition of minute duckweed grown in Maninjau Lake water

Figure 1 also shows a decline of duckweed growth with time, which can be explained in terms of nutrients depletion, particularly phosphate (Figure 4). The biomass productivity, however, tend to increase with time, which can be attributed to higher population density in the late stage of experiment. As has been previously reported that productivity is a function of biomass concentration and the specific growth rate [15], so that lower growth rate in late growth stage in this experiment was compensated by higher biomass density and resulted in higher biomass productivity. The duckweed productivity in this experiment was comparable with that in common carp fish water recirculation ponds [15], but lower than that was grown in a shaded closed water recirculation system [39]. It was also lower than duckweed grown in media of a eutrophic Saguling Reservoir water which was 161,11 g FW/m²/day [21]. Leng (1999) has reported that biomass productivity of various duckweed around the world ranged 2 –79 tons dry weight/ha/year [24], in which the productivity level more than 25 tons dry weight/ha/year was only obtained in cultures provided with optimum media and controlled

environment. Accordingly, the productivity level range in this experiment, in which the highest value was only 51 g FW/m²/day or equal to 11 ton DW/ha/year can be categorized into the medium level

Influence of ambient nutrient concentration was recorded during early growth stage (Figure 1), in which positive correlation between nutrient concentration with both duckweed growth rate and productivity was noticed. This phenomenon emphasizes that phosphorus limitation has inhibited minute duckweed growth in the late experimental stage.

3.3. Phytoremediation capacity

Duckweed capacity to control lake water contamination has been evaluated in terms of decreasing nutrient parameters in the rearing container water (Figure 3 & 4). There was a decrease of TSS, TIN, and TP average concentration from 13.50 - 19.50 mg/L, 0.126 - 0.182 mg/L and 0.053 - 0.061 mg/L in the starting day to 8.20 - 9.33 mg/L, 0.099 - 0.117 mg/L and 0.007 - 0.013 mg/L in day 9, which is equal to the average elimination rate of the plant on TSS, TIN, and TP of 39.26 - 57.26 %, 14.18 - 35.67 %, and 82.25 - 94.04 %, respectively. In consistent with this, there has also been a report the ability of minute duckweed to eliminate TIN and phosphate from Saguling Reservoir water as much as 74,05% and 73,36%, respectively [21], while Alaert *et al* (1996) reported 74 % TN and 77 % TP elimination rate of minute duckweed grown in a waste water treatment pond of 21 days retention time [23]. Comparing with these two previous reports, in Maninjau water the elimination rate of TIN was remarkable lower than that of phosphate and can also be means that phosphate became the major limiting factor for growth of the minute duckweed.

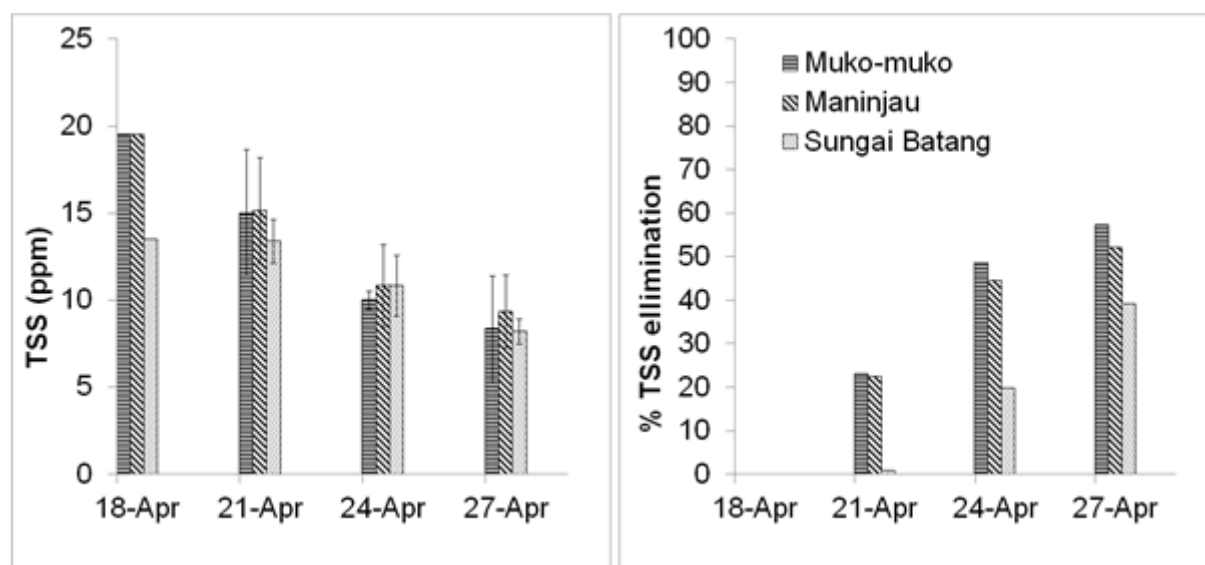


Figure 3. TSS elimination capacity of minute duckweed grown in Maninjau Lake water

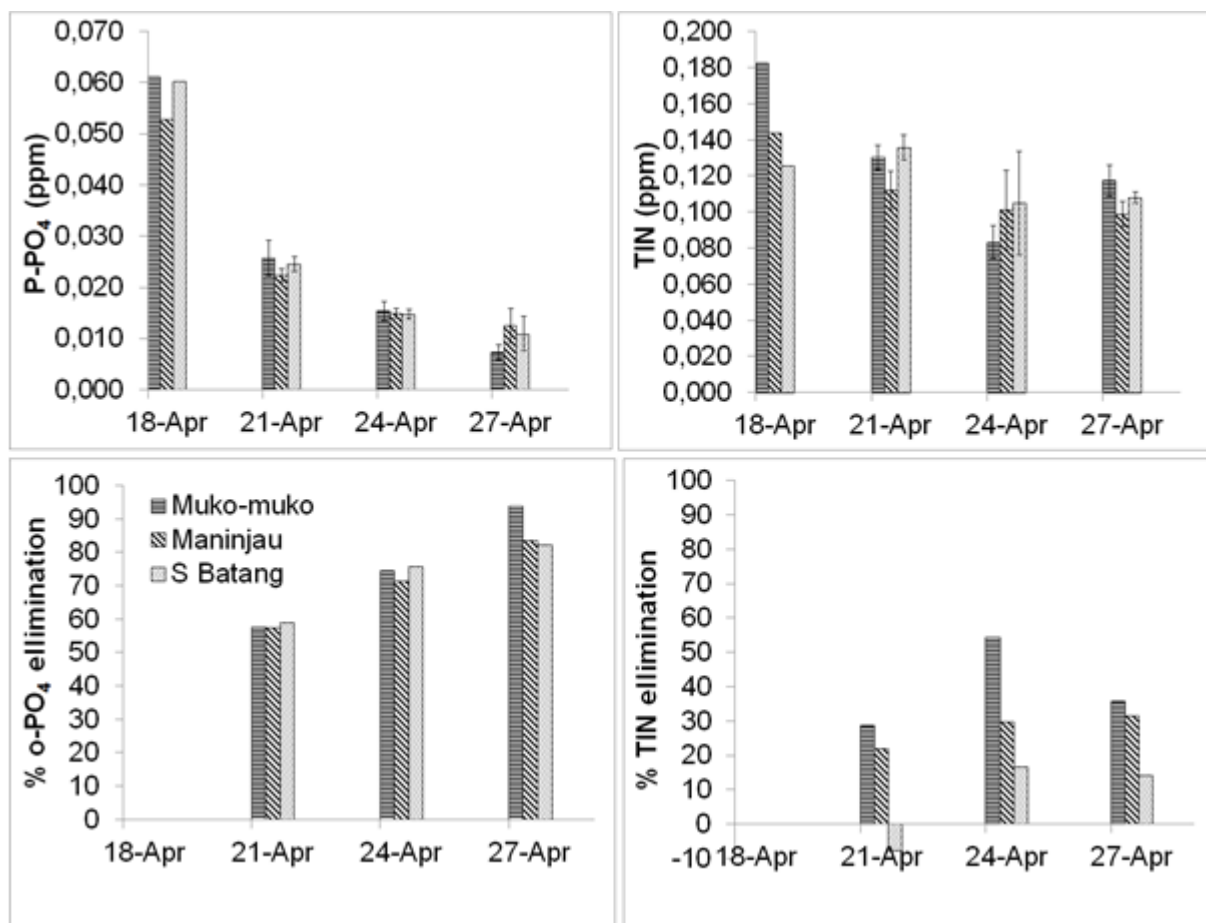


Figure 4. Phosphate and TIN ellimination capacity of minute duckweed grown in Maninjau Lake water

4. Conclusion

Results of this experiment shows that Maninjau Lake water did not vapor optimal growth of minute duckweed. Phosphate limitation and low temperature can be attributed to be the limiting growth factors. The duckweed growth, however, can eliminate as high as 94% phosphate and 54% total inorganic nitrogen from the water. Lower TIN elimination rate indicates that phosphate availability was the limiting factor for duckweed growth in the lake.

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