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Growth and Nutritional Values of *Moina micrura* Fed on *Chlorella vulgaris* Grown in Digested Palm Oil Mill Effluent

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Abstract

Moina micrura Kruz (Isolate no. AR001), a cladoceran and an important aquaculture feed, was cultured on *Chlorella vulgaris* Beijerinck (Isolate no. 001) grown in 10 and 20% digested palm oil mill effluent (POMED) and inorganic fertilizer (N:P:K = 1:1:0.50) in 70 l tanks. The collected microalgae was distributed to *M. micrura* twice daily at the rate of 3% dry body weight for 14 days. The specific growth rate of *M. micrura* was significantly higher ($p<0.05$) when fed on microalgae grown in 10% POMED than fed on the same cultured in 20% POMED and fertilizer. The protein and lipid contents were significantly higher ($p<0.05$) in *M. micrura* fed on microalgae grown in 10% POMED than in those fed on this microalgae cultured in other media. The essential amino acids such as histidine, arginine, threonine, phenylalanine, valine, methionine, lysine and tryptophan of *C. vulgaris* grown in 10% POMED enhanced the accumulation of these EAAs in *M. micrura*. The C18, C20 and C22 polyunsaturated fatty acids (PUFAs) viz. linolenic acid (18:3n-3), ϒ linolenic acid (18:3n-6), eicosatrienoic acid (20: 3n-6), arachidonic acid (20: 4n-6), EPA (20: 5n-3), DPA (22: 5n-3) and DHA (22: 6n-3) of *C. vulgaris* grown in 10% POMED enhanced the levels in *M. micrura*. The PUFAs were found significantly higher ($p<0.05$) in *M. micrura* fed on *C. vulgaris* cultured in 10% POMED followed by 20% POMED and only inorganic fertilizer. Most of the essential minerals were present in high amount in *M. micrura* fed on this algae grown in 20% and then 10% POMED followed by fertilizer. The characteristics of effluents

and mineral contents were reduced at least 10 to 12 times after growing *C. vulgaris*, which indicate the suitability of the supernatant of residues to release in the waterways. This study showed that the nutritional values of *M. micrura* were dependent on the nutritional values of the feed, *C. vulgaris*.

Introduction

The Malaysian palm oil industries produce large amounts of effluents every year (Phang 1987 and 1990; Habib et al. 1998). The effluents contain high levels of organic matter (Phang 1990; Habib et al. 1998) including colloidal protein, carbohydrates, nitrogenous compounds, lipids and minerals (Okiy 1987; Kekwick 1997; Habib et al. 1998) that can be utilized as nutrients by algae after degradation into inorganic forms through bacterial activities (Phang 1990; Chui 1993; Geetha et al. 1994; Habib et al. 1997).

Microralgae can bioaccumulate a long chain of unsaturated fatty acids (UFAs), amino acids, carotene and store minerals from the cultured media of heterotrophic and/or mixotrophic condition (Hirayama et al. 1989; Johns 1994; Habib et al. 1997; Vazhappilly and Chen 1998). The digested palm oil mill effluent (POMED) can act as the medium with organic and inorganic nutrients to fulfil the requirements of heterotrophic and mixotrophic microalgae like *Chlorella vulgaris* (Chui 1993; Anton et al. 1994; Geetha et al. 1994; Johns 1994). Zooplankton, an important natural food for fish, and an excellent source of essential amino acids (EAAs) and polyunsaturated fatty acids (PUFAs) cannot accumulate these essential micronutrients in significant amounts but their concentrations can be increased through consumption of the right kind of algae (Barclay et al. 1994). Among zooplankton, *M. micrura* is a commercially important cladoceran rich in nutrients making it an excellent live food for the good growth and development of fish and prawn larvae (Alam et al. 1991 and 1993).

Very few research reports regarding the culture of algae in digested POME and its use as feed for zooplankton and fish are available (Anton et al. 1994; Yusoff and Chan 1997; Habib et al. 1997). Therefore, the present work was undertaken to utilize digested POME to produce enriched microalgae *C. vulgaris*, fed to *M. micrura* to illustrate that we can improve the nutritional value of *M. micrura*.

Materials and Methods

The raw palm oil mill effluent was collected from Golden Hope Mill, Carry Island, Selangor, Malaysia and aerobically digested by aeration for 16 days to achieve a supernatant free of suspended solids. The physical and chemical properties such as pH, BOD, total dissolved solids (TDS), total suspended solids (TSS), total nitrogen, ammonia nitrogen and orthophosphate of media before setting the experiment were analyzed following the methods of Clesceri et al. (1989). The experiment was conducted in five treatments such as 5, 10, 20 and 30% of digested palm oil mill effluent (POMED), and control as commercial fertilizer (N:P:K = 1.0: 1.0: 0.50) in 70 l aerated tanks at the

Hatchery Complex of the Faculty of Fisheries and Marine Science, Universiti Putra Malaysia, Selangor, Malaysia. As the sources of nitrogen, phosphorus and potash, 260 mg·l^{-1} urea (119.6 mg N), 1.05 g·l triple super phosphate (119.60 mg P) and 154.64 mg·l^{-1} murite of potash (59.80 mg K) were used based on Habib et al. (1997) with little modifications. The experiment was conducted in triplicates. A microalgae, *C. vulgaris* isolated from Malaysian freshwater pond and cultured in Bold Basal medium (Tompkins et al. 1995) was used as inoculum when it reached optical density, OD_{620} = 0.20 (Cell No. 2.49-2.51 x 10^5·ml^{-1}). This microalga was collected from tanks just before the stationary phase. The algal samples were cleaned with deionized water through repeated centrifugation at 3000 rpm for 10 min before storing in the refrigerator at 4ºC. The refrigerated microalgae were allowed to return at ambient temperature (27 to 28ºC) before feeding to *M. micrura*. Ten mature *M. micrura* per liter were inoculated into 70 l aerated tanks containing 20 l water and were fed twice a day at the rate of 10 ml concentrated algae per tank (OD_{620} = 2.5-3.2, 1.115 to 1.12 g·l^{-1} dry biomass). *C. vulgaris* and *M. micrura* were counted using improved Neubauer haemacytometer and Sedgewick rafter counting chamber, respectively under the microscope on alternate days by taking 10 ml samples from the tanks to monitor growth. The feeding rate of *M. micrura* was adjusted accordingly. The optical density of algae was determined using UV-Spectrophotometer (Shimadzu UV 160) and pH, dissolved oxygen, total nitrogen and ammonia nitrogen were recorded daily following Clesceri et al. (1989). After 11 days, *M. micrura* was collected, cleaned with deionized water with repeated washings through centrifugation at 3000 rpm for 10 min and kept at 0°C for two days. Frozen *C. vulgaris* and *M. micrura* samples were kept for two days at -80°C before freeze drying. The samples were freeze dried at -50°C under vacuum pressure of 150 millitorr for two days and again stored at -80°C for further chemical analyses.

The proximate composition of stored samples was determined following the methods of Horwitz (1984). Crude protein by Kjeltec Auto 1030 Analyser and crude lipid by solvent extraction were determined. Carbohydrate (soluble) was analyzed by Phenol-sulphonic method (Kochart 1978). Amino acids of preserved samples were determined using Waters HPLC 5100 (Millipore 1990). Two mg of deproteinized samples were hydrolyzed with 0.50 ml 4.0 M methanesulfonic acid containing 0.20% tryptamine at 115°C for 24 hrs and cooled samples were diluted with 10 ml of 2.50 mmol·ml^{-1} L-α-amino-n-butyric acid as an internal standard and then filtered through 0.45 micro m Whatman filter paper. Amino acids were then detected after injection of 10-micro l sample at 37°C onto a single column Waters HPLC 501 unit with double pump (Millipore 1990) with 30 minutes retention time comparing with peaks of known standard as described by Habib et al. (1997). The lipids were collected from frozen samples after homogenizing with chloroform:methanol:water (1.0:2.0:0.80) following Folch et al. (1957). The fatty acid methyl esters (FAME) were obtained after hydrolysis of 10 mg lipid sample at 100°C for 1.5 hrs with 3.0 ml 14% boron trifluride-methanol following the methods of Benitez (1989). The fatty acids were determined after injection of 1.0 micro l FAME at 230°C onto a Thermon 3000 capillary column in Shimadzu GC-14A with 37 minutes retention time

and as described by Habib et al. (1997). The peaks were identified comparing with the peaks of known standards of fatty acids from Sigma Chemical Co. (Benitaz 1989). The fatty acids were then identified and quantified by Shimadzu GC-17A Mass Spectrometer (GC-MS) (QP-5000) using an Omegawax 320 capillary column: 30 m x 0.32 mm ID, 0.25 micro m film (Supelco) with a 25 cm·sec flow of Helium as the carrier gas.

The mineral content of samples was detected first by energy dispersive analysis of X-ray (EDAX) through scanning electron microscope (SEM) (Habib et al. 1997), and then detected and quantified by inductively coupled plasma Mass Spectrophotometer (ICP-MS) after digestion of 10 mg dry sample with 10 ml 6.0 N conc. nitric acid and 1.0 ml hydrogen peroxide except Sulphur (Habib et al. 1997). Sulphur was analyzed by Elemental Analyser after burning the sample at 670°C for 2 min and then detected and quantified by S-column (Perkin Elmer 1994). To compare treatment means of specific growth rates, total carotene, total biomass, protein, lipid, carbohydrate, essential amino acids, unsaturated fatty acids and essential minerals of *C. vulgaris* and *M. micrura*, one way ANOVA was performed using SAS computer package followed by Tukey test (Zar 1984).

Results and Discussions

The characteristics of the different dilutions of POMED are shown in table 1. Data indicate the richness of POMED in organic as well as inorganic nutrients with very little deficiency of nitrogen (Yusoff and Chan 1997; Habib et al. 1998). The C:N:P ratios were appropriate with the recommended ratio (56:9:1) for algal growth (Edwards et al. 1980). However, the specific growth rate (SGR) of *C. vulgaris* grown in 10% POMED was significantly higher ($p<0.05$) than that cultured in 5% POMED and fertilizer followed by 20 and 30% POMED (Table 2). Anton et al. (1994) obtained higher growth of *Ankistrodesmus convolutus* at 14% POMED followed by 10% POMED and then in 20 and 30% POMED which might be due to inhibition of photosynthesis by highly colored POMED (Phang and Ong 1988; Nedosekin et al. 1991). *Chlorella vulgaris* grew slowly in higher concentrations of POMED due to inadequate light in the dark

Table 1. Average (mean ± SE, n = 3) chemical contents ($mg.l^{-1}$ except pH) of different dilutions of digested palm oil mill effluent (POMED).

Parameters	5% POMED	10% POMED	20% POMED	30% POMED
PH	7.1 ± 0.10	6.9 ± 0.1	6.8 ± 0.1	6.6 ± 0.1
Dis. O_2	3.9 ± 0.10	3.8 ± 0.1	3.6 ± 0.1	3.5 ± 0.1
COD	1042.7 ± 12.8	2179.5 ± 25.6	4245.5 ± 75.7	6458.9 ± 65.1
TS	443.2 ± 5.1	975.7 ± 8.1	1926.0 ± 11.3	2844.3 ± 15.1
TSS	252.3 ± 3.0	524.5 ± 4.1	959.7 ± 6.1	1645.3 ± 8.1
Total N	58.5 ± 2.2	118.6 ± 3.3	228.0 ± 4.4	334.2 ± 5.4
NH_3-N	4.6 ± 0.2	8.9 ± 0.4	17.1 ± 0.8	29.6 ± 1.9
PO_4-P	8.4 ± 0.3	17.9 ± 0.5	34.20 ± 1.3	51.7 ± 2.3
C:N:P ratio	56.2: 8.4:1.2	56.04: 8.13:1.2	56.9: 8.0:1.2	56.3: 7.8:1.2

TS = Total Solids, TSS = Total Suspended Solids

colored medium (Phang and Ong 1988; Anton et al. 1994). Algae cultured in 5 and 30% POMED was not used to feed *M. micrura* because of poor algal growth. The alga cultured in these highly concentrated POMED took a long time to reach the stationary phase. Yusoff et al. (1996) reported that the diluted raw POME gave good growth of algae, which may be used to feed fish. In addition, the chlorophyll *a* content of *C. vulgaris* grown in 10% POMED was significantly higher ($p<0.05$) than that cultured in 20% POMED and fertilizer.

The specific growth rate of *M. micrura* fed on *C. vulgaris* grown in 10% POMED was found to be significantly higher ($p<0.05$) than those fed on the microalgae cultured in 20% POMED, followed by commercial fertilizer (Table 2). Protein (<50%) and lipid (<14%) contents were higher in *C. vulgaris* cultured in 10 and 20% POMED than fertilizer (Table 3) which in turn enhanced the protein and lipid contents of *M. micrura*. *M. micrura* fed on algae cultured in 10% POMED showed significantly higher ($p<0.05$) protein and lipid than that fed on algae grown in other media (Table 4). Similarly, Vikineswary (1994) found that *Chlorella vulgaris* contained higher protein and lipid when grown in palm oil mill effluent than cultured in other media.

Table 2. Specific growth rates (SGR) of *C. vulgaris* (algae) and *M. micrura* fed on *C. vulgaris*, chlorophyll *a* (Chl-*a*) and total biomass of *C. vulgaris* grown in 10% and 20% palm oil mill effluent (POMED), and control.

Parameters	*C. vulgaris* grown in 5% POMED	*C. vulgaris* grown in 10% POMED	*C. vulgaris* grown in 20% POMED	*C. vulgaris* grown in 30% POMED	*C. vulgaris* grown in control
SGR of cell	0.31 ± 0.03^{b}	0.40 ± 0.03^{a}	0.24 ± 0.02^{c}	0.17 ± 0.02^{c}	0.32 ± 0.02^{b}
Chlorophyll *a* (mg/l)	7.10 ± 0.30^{b}	14.71 ± 0.82^{a}	11.46 ± 0.65^{b}	11.25 ± 0.655^{b}	8.74 ± 0.45^{c}
SGR of chlorophyll- *a*	0.30 ± 0.03^{b}	0.37 ± 0.03^{a}	0.22 ± 0.02^{b}	0.19 ± 0.02^{b}	0.30 ± 0.02^{c}
Total biomass (Chl-*a* x 67*) mg/l	463.5 ± 8.2^{d}	985.6 ± 12.8^{a}	767.8 ± 10.6^{b}	753.8 ± 9.9^{b}	585.7 ± 8.2^{c}
		Fed on algae grown in 10% POMED	Fed on algae grown in 20% POMED		Fed on algae grown in control
SGR of *M. micrura*		0.78 ± 0.04^{a}	0.65 ± 0.04^{b}		0.50 ± 0.03^{c}

SGR = Specific Growth Rate, *Adan and Lee (1980), and Vonshak (1990)

SGR of cell and *M. micrura* = $\ln (X_2-X_1)/t_2-t_1$
where, X_2 = biomass concentration at the end of selected time interval;
X_1 = biomass concentration at the beginning of selected time interval; and
t_2-t_1 = elapsed time between selected time in day.

SGR of chlorophyll-a = $\ln (X_2-X_1)/t_2-t_1$
where, X_2 = Chlorophyll-a concentration at the end of selected time interval;
X_1 = Chlorophyll-a concentration at the beginning of selected time interval; and
t_2-t_1 = elapsed time between selected time in day.

Data on amino acid composition of *C. vulgaris* grown in 10 and 20% POMED, and fertilizer and *M. micrura* fed on these algae are reported in tables 5 and 6. Schwarz et al. (1995) reported that *M. micrura* fed on microalgae grown in pond contain all the EAAs. Among EAAs, histidine, arginine, threonine, phenylalanine, valine, methionine and lysine of *C. vulgaris* grown in 10% POMED were significantly higher ($p<0.05$) than those cultured in 20% POMED and fertilizer. Similarly, *M. micrura* fed with algae grown in 10% POMED contained significantly higher ($p<0.05$) amount of most of the EAAs except leucine, isoleucine and tryptophan than that fed on algae cultured in other media (Table 6).

The PUFAs, especially C18, C20 and C22 were significantly higher ($p<0.05$) in C. vulgaris cultured in 10% POMED than those grown in other media (Table 7). Chu et al. (1994) reported that these PUFAs were found to be higher in freshwater algae than marine ones. This study showed that *M. micrura* fed with *C. vulgaris* grown in 10% POMED had significantly higher ($p<0.05$) PUFAs than those fed with the same cultured in other media (Table 8). Nedosekin et al. (1991) reported that the variations in PUFAs depend on the availability of micronutrients as PUFAs in algae are grown in different media. Fatty acids with 20 and 22 carbon polyunsaturated fatty acids such as arachidonic acid (20:4n-6), eicosatrienoic acid (20: 3n-6), eicosapentaenoic acid (20:5n-3) and eicosahexaenoic acid (22: 6n-3) were found in *C. vulgaris* grown in PONED. These PUFAs were bioaccumulated in *M. micrura* though feeding of *C. vulgaris*. However, *C. vulgaris* do not synthesize C20 and C22 PUFAs

Table 3. Proximate composition (g·100 g dry sample) of *C. vulgaris* grown in 10 and 20% palm oil mill effluent (POMED), and control as N:P:K.

Proximate composition	*C. vulgaris* grown in 10% POMED	*C. vulgaris* grown in 20% POMED	*C. vulgaris* grown in control (N:P:K)
Moisture	6.6 ± 0.03^{a}	6.6 ± 0.03^{a}	6.6 ± 0.03^{a}
Crude protein	28.7 ± 0.19^{a}	24.4 ± 0.16^{b}	22.5 ± 0.15^{c}
Crude lipid	18.3 ± 0.13^{a}	16.8 ± 0.11^{b}	15.2 ± 0.10^{c}
Ash	15.9 ± 0.12^{b}	18.5 ± 0.11^{a}	13.5 ± 0.11^{c}
Carbohydrate	26.1 ± 0.18^{c}	28.1 ± 0.17^{b}	30.8 ± 0.22^{a}
NFE	4.3 ± 0.06^{c}	5.4 ± 0.06^{b}	12.5 ± 0.05^{a}

NFE = Nitrogen Free Extract, Means (± SE, n = 9) of with different superscripts in each row indicate significant differences ($P < 0.05$, df. = 23, 3)

Table 4. Proximate Composition (g·100 g dry sample) of *M. micrura* fed on *C. vulgaris* grown in 10 and 20% palm oil mill effluent (POMED), and control.

Proximate composition	Fed on *C. vulgaris* grown in 10% POMED	Fed on *C. vulgaris* grown in 20% POMED	Fed on *C. vulgaris* grown in control
Moisture	6.7 ± 0.03^{a}	6.7 ± 0.03^{a}	6.7 ± 0.03^{a}
Crude protein	56.1 ± 0.25^{a}	53.7 ± 0.23^{b}	50.5 ± 0.22^{c}
Crude lipid	19.5 ± 0.13^{a}	17.6 ± 0.11^{b}	16.7 ± 0.12^{c}
Ash	16.3 ± 0.12^{b}	20.6 ± 0.15^{a}	15.5 ± 0.12^{c}
NFE	1.3 ± 0.04^{b}	1.3 ± 0.04^{b}	8.2 ± 0.10^{a}

NFE = Nitrogen Free Extract, Means (± SE, n = 9) with different superscripts in each row indicate significant differences ($P < 0.05$, df. 23, 3)

except in very small amount (Oka et al. 1982; Chu et al. 1994). Chui (1993) and Johns (1994) have reported that *C. vulgaris* can accumulate a long chain of PUFAs when grown in heterotrophic media such as POMED.

Table 5. Amino Acids (g·100 g protein) of *C. vulgaris* cultured in 10 and 20% palm oil mill effluent (POMED), and control.

Amino acids	*C. vulgaris* grown in 10% POMED	*C. vulgaris* grown in 20% POMED	*C. vulgaris* grown in control
Aspartic acid	9.4 ± 0.12	8.5 ± 0.11	9.7 ± 0.11
Glutamic acid	7.9 ± 0.10	7.6 ± 0.14	8.8 ± 0.10
Serine	8.9 ± 0.11	9.5 ± 0.13	9.4 ± 0.12
Glycine	6.2 ± 0.06	7.2 ± 0.10	10.5 ± 0.16
Histidine*	3.5 ± 0.06^{a}	3.0 ± 0.05^{b}	2.2 ± 0.04^{c}
Arginine*	5.6 ± 0.12^{a}	4.9 ± 0.10^{b}	3.4 ± 0.06^{c}
Threonine*	6.2 ± 0.10^{a}	5.1 ± 0.08^{b}	4.1 ± 0.05^{c}
Alanine	8.2 ± 0.11	9.4 ± 0.12	12.8 ± 0.13
Proline	4.6 ± 0.06	6.1 ± 0.10	8.5 ± 0.10
Tyrosine*	3.1 ± 0.04^{b}	3.2 ± 0.04^{b}	3.5 ± 0.05^{a}
Phenylalanine*	3.5 ± 0.05^{a}	3.3 ± 0.05^{b}	2.0 ± 0.04^{c}
Valine*	6.8 ± 0.09^{a}	6.2 ± 0.08^{b}	5.8 ± 0.07^{c}
Methionine*	5.6 ± 0.10^{a}	4.4 ± 0.07^{b}	3.0 ± 0.04^{c}
Cystine	0.9 ± 0.02	0.9 ± 0.02	2.3 ± 0.04
Isoleucine*	3.2 ± 0.03^{a}	2.9 ± 0.03^{a}	2.2 ± 0.03^{b}
Leucine*	4.2 ± 0.08^{b}	4.7 ± 0.07^{a}	4.0 ± 0.07^{b}
Lysine*	6.1 ± 0.09^{a}	5.3 ± 0.04^{b}	3.5 ± 0.03^{c}
Tryptophan*	1.7 ± 0.04^{a}	1.6 ± 0.03^{a}	1.1 ± 0.03^{b}

Means (± SE, n = 9) of *essential amino acid with different superscripts in each row indicate significant differences ($P < 0.05$, df. 23, 3). The recovery of total amino acids varied from 94.74 to 95.87% based on total protein

Table 6. Amino Acids (g·100 g protein) of *M. micrura* fed on *C. vulgaris* cultured in 10 and 20% palm oil mill effluent (POMED), and control.

Amino acids	*M. micrura* fed on *C. vulgaris* grown in 10% POMED	*M. micrura* fed on *C. vulgaris* grown in 20% POMED	*M. micrura* fed on *C. vulgaris* grown in control
Aspartic acid	8.1 ± 0.07	9.4 ± 0.08	10.0 ± 0.11
Glutamic acid	6.5 ± 0.05	7.8 ± 0.07	8.2 ± 0.07
Serine	5.8 ± 0.07	6.7 ± 0.06	9.1 ± 0.08
Glycine	7.3 ± 0.06	8.2 ± 0.07	7.6 ± 0.06
Histidine*	4.7 ± 0.05^{a}	4.1 ± 0.04^{b}	2.9 ± 0.04^{c}
Arginine*	5.7 ± 0.05^{a}	5.2 ± 0.05^{b}	3.8 ± 0.04^{c}
Threonine*	4.6 ± 0.04^{a}	3.4 ± 0.03^{b}	2.5 ± 0.03^{c}
Alanine	6.1 ± 0.05	7.1 ± 0.06	11.6 ± 0.12
Proline	5.7 ± 0.06	6.1 ± 0.06	8.7 ± 0.07
Tyrosine*	4.6 ± 0.04^{a}	4.1 ± 0.04^{b}	4.1 ± 0.05^{b}
Phenylalanine*	4.6 ± 0.04^{a}	3.9 ± 0.05^{b}	2.1 ± 0.04^{c}
Valine*	5.9 ± 0.05^{a}	5.5 ± 0.05^{b}	5.6 ± 0.07^{b}
Methionine*	7.8 ± 0.06^{a}	6.3 ± 0.06^{b}	4.1 ± 0.04^{c}
Cystine	0.8 ± 0.02	0.8 ± 0.02	1.1 ± 0.03
Isoleucine*	3.7 ± 0.03^{a}	3.6 ± 0.03^{a}	2.4 ± 0.03^{b}
Leucine*	4.8 ± 0.04^{a}	4.9 ± 0.07^{a}	4.6 ± 0.05^{b}
Lysine*	6.9 ± 0.06^{a}	5.8 ± 0.04^{b}	3.7 ± 0.03^{c}
Tryptophan*	1.9 ± 0.04^{a}	1.9 ± 0.03^{a}	1.4 ± 0.03^{b}

Means (± SE, n = 9) of *essential amino acid with different superscripts in each row indicate significant differences ($P < 0.05$, df. 23, 3). The recovery of total amino acids varied from 93.13 to 95.11% based on total protein

Zooplankton and fish absorb some of the available dissolved minerals in water through the gill and intestinal wall (Lovell 1989) and major portion through food (Habib et al. 1997). In this study, *C. vulgaris* grown in 20% POMED contained a higher amount of most of the essential minerals than that grown in 10% POMED and fertilizer (Tables 9 and 10). Similarly, *M.*

Table 7. Fatty Acids (g·100 g lipid) of *C. vulgaris* grown in 10 and 20% palm oil mill effluent (POMED), and fertilizer.

Fatty acids	*C. vulgaris* grown in 10% POMED	*C. vulgaris* grown in 20% POMED	*C. vulgaris* grown in control
Lauric acid (12:0)	5.2 ± 0.25	7.4 ± 0.35	9.2 ± 0.43
Myristic acid (14:0)	6.6 ± 0.33	8.7 ± 0.50	8.7 ± 0.31
Palmitic acid (16:0)	8.3 ± 0.49	10.2 ± 0.72	16.5 ± 0.84
Heptadecanoic acid (17:0)	1.1 ± 0.04	1.1 ± 0.03	7.4 ± 0.32
10-heptadecanoic acid (17:01)*	1.6 ± 0.02^{a}	1.6 ± 0.03^{a}	1.1 ± 0.02^{b}
Stearic acid (18:0)	8.4 ± 0.43	11.2 ± 0.84	14.4 ± 0.92
Oleic acid (18:1n-9)*	5.0 ± 0.23^{c}	8.9 ± 0.47^{a}	5.7 ± 0.22^{b}
Linoleic acid (18:2n-6)*	7.9 ± 0.34^{a}	6.4 ± 0.26^{b}	6.7 ± 0.35^{b}
Linolenic acid (18:3n-3)**	15.7 ± 0.93^{a}	9.4 ± 0.58^{c}	13.3 ± 0.64^{b}
ϒ-Linolenic acid (18: 3n-6)**	2.2 ± 0.03^{a}	1.3 ± 0.03^{b}	0.6 ± 0.02^{c}
Arachidic acid (20:0)	4.5 ± 0.22	5.2 ± 0.24	2.2 ± 0.12
Eicosatrienoic acid (20:3n-6)**	3.3 ± 0.15^{a}	2.6 ± 0.09^{b}	0^{c}
Arachidonic acid (20:4n-6)**	4.1 ± 0.22^{a}	3.3 ± 0.17^{b}	0^{c}
Eicosapentaenoic acid (20:5n-3)**	5.2 ± 0.25^{a}	2.8 ± 0.13^{b}	0^{c}
Behenic acid (22: 0)	2.2 ± 0.04	3.1 ± 0.16	0
Docosapentaenoic acid (22: 5n-3)**	3.7 ± 0.06^{a}	2.3 ± 0.07^{b}	0^{c}
Docosahexaenoic acid (22: 6n-3)**	2.2 ± 0.04^{a}	1.9 ± 0.04^{b}	0^{c}

Means (± SE, n = 9) of *unsaturated and **polyunsaturated fatty acids with different superscripts in each row indicate significant differences ($P < 0.05$, df. 23, 3)

Table 8. Fatty Acids (g/100 g lipid) of *M. micrura* fed on *C. vulgaris* grown in 10 and 20% digested palm oil mill effluent (POMED), and fertilizer.

Fatty acids	*M. micrura* fed on algae grown in 10% POMED	*M. micrura* fed on algae grown in 20% POMED	*M. micrura* fed on algae grown in control
Capric acid (10:0)	4.2 ± 0.17	5.3 ± 0.24	7.1 ± 0.26
Lauric acid (12:0)	5.4 ± 0.22	5.4 ± 0.26	6.3 ± 0.24
Myristic acid (14:0)	6.1 ± 0.35	7.3 ± 0.46	8.5 ± 0.37
Palmitic acid (16:0)	5.2 ± 0.24	6.1 ± 0.35	6.3 ± 0.32
Heptadecanoic acid (17:0)	3.6 ± 0.12	4.6 ± 0.14	5.1 ± 0.22
10-heptadecanoic acid (17:01)*	1.4 ± 0.05	1.5 ± 0.03	1.9 ± 0.03
Stearic acid (18:0)	5.7 ± 0.23	7.9 ± 0.47	9.7 ± 0.52
Oleic acid (18:1n-9)*	3.5 ± 0.14^{b}	4.2 ± 0.24^{a}	4.1 ± 0.25^{a}
Linoleic acid (18:2n-6)*	6.4 ± 0.42^{b}	7.1 ± 0.36^{a}	7.3 ± 0.42^{a}
Linolenic acid (18:3n-3)**	20.2 ± 1.33^{a}	18.5 ± 1.05^{b}	15.3 ± 1.05^{c}
ϒ-Linolenic acid (18: 3n-6)**	2.3 ± 0.10^{a}	1.6 ± 0.08^{b}	0.9 ± 0.04^{c}
Arachidic acid (20:0)	4.7 ± 0.24	4.1 ± 0.24	3.9 ± 0.15
Eicosatrienoic acid (20:3n-6)**	3.6 ± 0.20^{a}	2.8 ± 0.20^{b}	1.3 ± 0.02^{c}
Arachidonic acid (20:4n-6)**	4.3 ± 0.23^{a}	3.5 ± 0.23^{b}	2.1 ± 0.11^{c}
Eicosapentaenoic acid (20:5n-3)**	5.4 ± 0.24^{a}	3.8 ± 0.17^{b}	2.4 ± 0.13^{c}
Behenic acid (22:0)	2.2 ± 0.13	2.1 ± 0.07	2.1 ± 0.10
Docosapentaenoic acid (22: 5n-3)**	3.9 ± 0.14^{a}	2.7 ± 0.16^{b}	1.8 ± 0.08^{c}
Docosahexaenoic acid (22: 6n-3)**	3.7 ± 0.13^{a}	2.6 ± 0.15^{b}	2.1 ± 0.09^{c}

Means (± SE, n = 9) of *unsaturated and **polyunsaturated fatty acids with different superscripts in each row indicate significant differences ($P < 0.05$, df. 23, 3)

Table 9. Mineral content (mg·1 fresh sample) of 10, 20% digested palm oil mill effluent POMED, and N:P:K

Minerals	10% POMED	20% POMED	N: P : K
Fe*	7.9 ± 0.11	10. 4 ± 0.16	3.3 ± 0.14
Zn*	4.8 ± 0.03	7. 1 ± 0.05	0.3 ± 0.03
P*	255.2 ± 1.12	514. 9 ± 1.55	22.3 ± 3.22
Na*	6.9 ± 0.07	13. 8 ± 0.15	6.8 ± 0.45
Mg*	8.7 ± 0.11	18.3 ± 0.21	1.7 ± 0.07
Mn*	0.9 ± 0.01	1.8 ± 0.03	0.1 ± 0.01
Ca*	53.1 ± 0.33	105.3 ± 0.78	11.3 ± 0.55
K*	96.3 ± 0.32	192.2 ± 1.25	117.9 ± 0.36
Co*	0.1 ± 0.01	0.2 ± 0.01	0.02 ± 0.001
Cr*	0.6 ± 0.04	1.3 ± 0.06	0.1 ± 0.003
Cu*	0.8 ± 0.04	1.4 ± 0.07	0.4 ± 0.02
Ni*	0.2 ± 0.02	0.3 ± 0.03	0.03 ± 0.001
S*	3.9 ± 0.05	5.3 ± 0.08	0.04 ± 0.004
Se*	0.7 ± 0.04	1.5 ± 0.06	0.02 ± 0.001
Si*	0.5 ± 0.03	0.9 ± 0.06	8.5 ± 0.55
Al*	13.6 ± 0.08	22.3 ± 0.15	1.4 ± 0.15
B*	1.1 ± 0.05	2.6 ± 0.07	0.3 ± 0.02
Mo*	0.3 ± 0.02	0.6 ± 0.04	0
Sn*	2.4 ± 0.04	4.6 ± 0.08	0.04 ± 0.001
V*	0.2 ± 0.01	0.3 ± 0.02	0.02 ± 0.001
As*	0.3 ± 0.03	0.6 ± 0.04	0.2 ± 0.03
Li*	0.21 ± 0.02	0.3 ± 0.03	0.10 ± 0.01
Pb*	0.25 ± 0.02	0.5 ± 0.03	0.4 ± 0.03
Cd	0.03 ± 0.003	0.1 ± 0.003	0.1 ± 0.001

*Essential mineral

Table 10. Mineral content (micro $g \cdot g^{-1}$ sample on dry weight basis) of *C. vulgaris* grown in 10%, 20% palm oil mill effluent (POMED), and control detected and quantified by ICP-MS.

Minerals	10% POMED	20% POMED	Control (N:P:K)
Fe*	28.2 ± 1.30^{b}	56.2 ± 2.20^{a}	15.1 ± 0.23^{c}
Zn*	24.1 ± 1.01^{b}	47.5 ± 2.05^{a}	4.8 ± 0.05^{c}
P*	4135.0 ± 152.02^{b}	7500.5 ± 186.15^{a}	535.1 ± 5.65^{c}
Na*	53.2 ± 1.31^{c}	106.4 ± 2.70^{a}	76.4 ± 1.80^{b}
Mg*	75.5 ± 3.14^{b}	153.7 ± 5.35^{a}	20.7 ± 1.40^{c}
Mn*	10.2 ± 0.11^{b}	18.8 ± 0.30^{a}	1.1 ± 0.04^{c}
K*	442.3 ± 10.25^{c}	823.3 ± 6.04^{a}	693.3 ± 6.85^{b}
Ca*	742.6 ± 13.15^{b}	1336.5 ± 25.38^{a}	160.6 ± 2.90^{c}
Co*	1.7 ± 0.02^{b}	3.3 ± 0.03^{a}	0.1 ± 0.01^{c}
Cr*	8.5 ± 0.32^{b}	15.7 ± 0.84^{a}	0.9 ± 0.05^{c}
Cu*	11.8 ± 0.70^{b}	22.2 ± 1.04^{a}	4.9 ± 0.14^{c}
Ni*	1.4 ± 0.05^{b}	2.6 ± 0.08^{a}	0.5 ± 0.04^{c}
S*	31.8 ± 2.23^{b}	62.5 ± 3.80^{a}	0.7 ± 0.05^{c}
Se*	9.8 ± 0.77^{b}	17.3 ± 0.60^{a}	0.3 ± 0.03^{c}
Si*	0	0	0
Al*	36.4 ± 2.22^{b}	73.8 ± 3.12^{a}	9.9 ± 0.21^{c}
B*	13.7 ± 0.64^{b}	25.3 ± 1.05^{a}	4.7 ± 0.05^{c}
Mo*	4.8 ± 0.15^{b}	8.9 ± 0.36^{a}	0.1 ± 0.01^{c}
Sn*	6.4 ± 0.51^{b}	11.6 ± 0.74^{a}	0.5 ± 0.03^{c}
V*	1.3 ± 0.04^{b}	2.5 ± 0.06^{a}	0.2 ± 0.01^{c}
As*	5.5 ± 0.08^{b}	10.3 ± 0.24^{a}	2.2 ± 0.02^{c}
Li*	0.2 ± 0.02^{b}	0.5 ± 0.03^{a}	0.1 ± 0.01^{c}
Pb*	2.5 ± 0.05^{b}	4.5 ± 0.13^{a}	2.9 ± 0.28^{c}
Cd	0.3 ± 0.03^{b}	0.6 ± 0.03^{a}	0.2 ± 0.02^{c}
Total	5647.2	10304.5	1535.3

*Essential mineral

micrura bioaccumulated higher amounts of essential minerals fed on algae grown in 20% POMED followed by 10% POMED and fertilizer (Table 11). Schwarz et al. (1995) stated that zooplankton accumulates minerals in proportion to their availability in the aquatic habitats. Silica was not detected in *C. vulgaris* grown in all the media, but it was found in *M. micrura* (Table 11). It is assumed that *M. micrura* bioaccumulated Si from another source such as water. Mineral contents of *M. micrura* (Table 11) was almost four times higher than those of *C. vulgaris* (Table 10). The chemical contents of supernatant of diluted effluents used to grow *C. vulgaris* were at least 10 times lower than the same of diluted effluents before the experiment (Table 12). Similarly,

Table 11. Mineral content (micro $g \cdot g^{-1}$ sample on dry weight basis) of *M. micrura* fed on *C. vulgaris* grown in 10%, 20% palm oil mill effluent (POMED), and fertilizer detected and quantified by ICP-MS.

Minerals	10% POMED	20% POMED	Fertilizer (N:P:K)
Fe*	91.20 ± 2.65^b	102.35 ± 2.78^a	79.55 ± 2.33^c
Zn*	105.35 ± 2.51^b	125.15 ± 2.85^a	32.60 ± 0.45^c
P*	14135.02 ± 365.15^b	18235.35 ± 484.12^a	10120.35 ± 205.86^c
Na*	780.25 ± 50.35^c	1012.05 ± 55.65^a	672.15 ± 33.20^b
Mg*	685.60 ± 35.10^b	945.30 ± 41.25^a	355.25 ± 18.15^c
Mn*	40.05 ± 1.15^b	69.45 ± 1.55^a	18.12 ± 0.75^c
K*	1855.20 ± 82.15^c	2525.15 ± 145.15^a	2235.45 ± 155.75^b
Ca*	10655.15 ± 296.30^b	13132.25 ± 315.30^a	625.50 ± 30.18^c
Co*	1.78 ± 0.02^b	2.18 ± 0.03^a	0.08 ± 0.01^c
Cr*	77.20 ± 3.20^b	88.45 ± 3.55^a	15.75 ± 1.02^c
Cu*	42.35 ± 3.75^b	60.20 ± 4.15^a	12.65 ± 0.85^c
Ni*	8.20 ± 0.42^b	13.40 ± 0.65^a	4.58 ± 0.25^c
S*	105.65 ± 3.55^b	133.55 ± 5.75^a	6.85 ± 0.35^c
Se*	78.45 ± 3.85^b	97.35 ± 3.97^a	4.15 ± 0.35^c
Si*	5.15 ± 0.33^b	8.70 ± 0.45^a	3.60 ± 0.23^c
Al*	75.05 ± 3.35^b	115.05 ± 4.60^a	43.25 ± 2.45^c
B*	42.30 ± 2.55^b	65.40 ± 3.35^a	18.15 ± 0.77^c
Mo*	25.75 ± 1.82^b	37.66 ± 2.14^a	0.55 ± 0.03^c
Sn*	47.55 ± 2.55^b	62.35 ± 3.88^a	8.47 ± 4.15^c
V*	7.25 ± 0.55^b	11.05 ± 0.85^a	1.25 ± 0.04^c
As*	10.50 ± 0.35^c	14.75 ± 0.54^a	12.15 ± 0.75^b
Li*	2 .15 ± 0.15^b	3.25 ± 0.25^a	0.97 ± 0.04^c
Pb*	5.25 ± 0.35^b	6.05 ± 0.45^a	4.75 ± 0.25^c
Cd	0.35 ± 0.02	0.65 ± 0.04	0.69 ± 0.03
Total	28882.75	36867.09	14276.86
% of body weight	2.89	3.69	1.43

*Essential mineral

Table 12. Average (mean ± SE) chemical contents ($mg.l^{-1}$ except pH) of 10 and 20% digested palm oil mill effluent (POMED) after experiment.

Parametres	10% POMED	20% POMED
pH	7.3 ± 0.25	7.2 ± 0.20
Dissolved Oxygen	3.7 ± 0.15	3.5 ± 0.12
Chemical Oxygen Demand	180.6 ± 5.2	220.6 ± 6.3
Total Solids	75.4 ± 4.7	115.4 ± 6.2
Total Suspended Solids	32.2 ± 1.5	42.5 ± 1.6
Total Nitrogen	10.6 ± 0.5	17.6 ± 0.8
Ammoniacal Nitrogen (NH3-N)	0.6 ± 0.05	0.8 ± 0.07
Orthophosphate (PO4-P)	1.5 ± 0.3	2.8 ± 0.5

Table 13. Mineral contents (mg·1 fresh sample) of 10%, 20% and N:P:K.

Minerals	10% POMED	20% POMED	N: P : K
Fe*	1.5 ± 0.1	1. 9 ± 0.1	1.2 ± 0.1
Zn*	1.9 ± 0.03	3.2 ± 0.1	0
P*	5.5 ± 0.2	11. 4 ± 0.3	2.0 ± .2
Na*	2.6 ± 0.07	4. 7 ± 0.2	0.6 ± 0.02
Mg*	1.0 ± 0.1	1.6 ± 0.15	0
Mn*	0.2 ± 0.0	0.3 ± 0.03	0
Ca*	10.4 ± 0.4	15.2 ± 0.6	1.1 ± 0.02
K*	7.6 ± 0.3	13.5 ± 0.3	7.3 ± 0.2
Co*	0	0	0
Cr*	0	0	0
Cu*	0	0	0
Ni*	0	0	0
S*	0	0	0
Se*	0	0	0
Si*	0.4 ± 0.03	0.8 ± 0.05	7.8 ± 0.3
Al*	6.2 ± 0.05	12.1 ± 0.2	1.0 ± 0.01
B*	0	0	0
Mo*	0	0	0
Sn*	1.2 ± 0.04	4.6 ± 0.08	0
V*	0.1 ± 0.01	0.3 ± 0.02	0
As*	0.1 ± 0.03	0.6 ± 0.04	0
Li*	0.2 ± 0.02	0.3 ± 0.03	0.08 ± 0.01
Pb*	0.1 ± 0.02	0.5 ± 0.03	0.20 ± 0.02
Cd	0.01 ± 0.003	0.1 ± 0.003	0.02 ± 0.001

*Essential mineral

mineral contents of supernatant of diluted effluents after experiment were at least 10 to 12 times lower than those of effluent used before the experiment (Table 13). These two results of the experiment indicate the suitability of the supernatant to release almost harmlessly in the waterways.

Conclusion

Algae enriched with EAAs, PUFAs and essential minerals can be obtained by culturing them in 10% digested POME due to availability of nutrients, appropriate color and proper light penetration in the medium. This microalgae in turn forms excellent live feed for zooplankton and fish. This study illustrated that the nutritional value of *M. micrura* can be improved through the use of POMED at 10%. It is also concluded that the supernatant of the diluted effluents is suitable to release in waterways due to its at least 10 to 12 times lower chemical and mineral contents than those before using to grow *C. vulgaris*.

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