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Assessment of three Tropical Chlorophytes as Bioassay Organisms for Nitrogen and Phosphorus Enrichment in Freshwater Ecosystems

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Abstract Enrichment of nitrogen and phosphorus in aquatic ecosystems can lead to excessive blooming of algae, resulting in eutrophication. The main objective of the present study was to assess the potential use of three tropical chlorophytes, namely *Chlorella vulgaris* UMACC 001, *Scenedesmus quadricauda* UMACC 041 and *Ankistrodesmus convolutus* UMACC 101 as test organisms for the bioassay of nitrogen and phosphorus. The minimal medium used in this study was 1% Bold's Basal Medium (BBM), which contained 0.03 mM $NaN0_3$ or NH_4Cl and 0.02 mM phosphate (KH_2PO_4 and K_2HPO_4). The algae were grown in the minimal medium added with $NaNO_3$ or NH_4Cl ranging from 0.03, 0.15, 0.75, 3.75 to 18.75 mM for 96 hours using flask cultures. The dilution water without nitrogen and phosphorus was used as the control. For the phosphate experiments, the cultures were grown at 0, 0.02, 0.10, 0.50, 2.50 and 12.50 mM phosphate (KH_2PO_4 and K_2HPO_4) in 1% BBM containing 0.03 mM $NaNO_3$ or NH_4Cl. There was no marked difference in the specific growth rates (μ) of the three chlorophytes in response to nitrogen and phosphorus enrichment. The percentage growth enhancement (PGE_{96}) based on the percentage increase of cell number at 96 hours compared to that attained in dilution water was a useful parameter for the bioassay of nitrogen and phosphorus. The suitability of the algae as test organisms was assessed based on the linearity of the relationship between PGE_{96} and nitrogen and phosphorus concentration, as indicated by the regression coefficient (R^2). It was also based on the EC_{50}, which was the effective concentration that gave a PGE_{96} of 50%. Based on the two parameters, *Chlorella vulgaris* was found to be a suitable test organism for the bioassay of $NaNO_3$ (EC_{50} = 0.56 mM; R^2 = 0.98) while *Ankistrodesmus convolutus* was suitable for the bioassay of NH_4Cl (EC_{50} = 0.005; r^2= 0.86). When grown on $NaNO_3$, the sensitivity of *Chlorella vulgaris* to phosphate enrichment was very low (EC_{50} = 822.72 mM). In comparison, when grown on NH_4Cl, the three algae were sensitive to phosphate enrichment (EC_{50} = 0.08 – 0.12 mM). The three algae would be useful for the bioassay of phosphate in water samples containing NH_4Cl as the dominant nitrogen source.

Abstrak Penambahan nitrogen dan fosforus ke dalam ekosistem akuatik boleh menyebabkan eutrofikasi akibat daripada ledakan pertumbuhan alga. Objektif utama kajian ini adalah untuk menentukan samada tiga alga hijau (klorofit), *Chlorella vulgaris* UMACC 001, *Scenedesmus quadricauda* UMACC 041 dan *Ankistrodesmus convolutus* UMACC 101 boleh digunakan untuk bioasai nitrogen dan fosforus. Medium minimum yang digunakan dalam kajian ini adalah 1% Bold Basal Medium (BBM) dan ia mengandungi 0.03 mM $NaNO_3$ atau NH_4Cl dan 0.02 mM fosfat (KH_2PO_4 dan K_2HPO_4). Alga-alga tersebut ditumbuhkan dalam medium minimum dengan tambahan $NaNO_3$ atau NH_4Cl (0.03, 0.15, 0.75, 3.75 dan 18.75 mM) selama 96 jam. "Dilution water" tanpa nitrogen dan fosforus merupakan kawalan untuk kajian ini. Untuk eksperimen fosfat, kultur-kultur tersebut ditumbuhkan dalam pelbagai kepekatan fosfat (0, 0.02, 0.10, 0.50, 2.50 dan 12.50 mM) dalam 1% BBM mengandungi 0.03 mM $NaNO_3$ atau NH_4Cl. Keputusan menunjukkan tiada perbezaan ketara dari segi kadar pertumbuhan spesifik (μ) alga-alga tersebut terhadap tambahan nitrogen dan fosforus. Peratus pertambahan pertumbuhan (percentage growth enhancement) yang berasaskan peratus peningkatan bilangan sel pada 96 jam (PGE_{96}) berbanding dengan bilangan sel kultur dalam "dilution water"

merupakan parameter yang berguna untuk bioasai nitrogen dan fosforus. Kesesuaian alga-alga tersebut sebagai organisma ujian untuk bioasai adalah berasaskan korelasi linear antara PGE_{96} dan kepekatan nitrogen dan fosforus (koeffisien regresi, R^2). Ia juga bergantung kepada EC_{50}, yang merupakan kepekatan nitrogen dan fosforus yang dapat meningkatkan PGE_{96} sebanyak 50%. *Chlorella vulgaris* merupakan organisma yang sesuai untuk bioasai $NaNO_3$ (EC_{50} = 0.56 mM, R^2 = 0.96) manakala *Ankistrodesmus convolutus* adalah sesuai untuk bioasai NH_4Cl (EC_{50} = 0.005 mM, R^2 = 0.86). Sensitiviti *Chlorella vulgaris* terhadap penambahan fosfat adalah sangat rendah (EC_{50} = 822.72 mM) bila ditumbuhkan dalam medium mengandungi $NaNO_3$. Ketiga-tiga alga tersebut adalah sensitif terhadap fosfat (EC_{50} = 0.08 – 0.12 mM) jika ditumbuhkan dalam medium mengandungi NH_4Cl. Ini menunjukkan alga-alga tersebut adalah berguna untuk bioasai fosfat jika NH_4Cl merupakan komponen nitrogen utama dalam sampel air yang diuji.

(Algae, bioassay, nitrogen, phosphorus, eutrophication)

INTRODUCTION

Nitrogen and phosphorus are two of the most important elements in algae, contributing 0.78 - 11.25% and 0.04 – 7.98% of their dry weight respectively [1]. Discharge of wastewater rich in these two nutrients into the aquatic ecosystems can induce excessive algal growth, resulting in eutrophication [2, 3]. The development of algal blooms can lead to anoxia and toxic or harmful impacts on fisheries resources, ecosystems, and human health or recreation [3]. For instance, ingestion of nitrates and nitrites from polluted drinking water can induce methhaemoglobinaemia, hampering the transport of oxygen in humans [4]. According to the Malaysian Environmental Quality Report [5], a total of 93 rivers are polluted in terms of ammoniacal-nitrogen due to livestock farming and domestic waste.

In freshwater environments, phosphorus is often the most limiting nutrient for algal growth while in marine waters primary production is limited by nitrogen [3]. The upper limits of total nitrogen (TN) and total phosphorus (TP) for temperate eutrophic lakes are 1260 μg TN L^{-1} and 71 μg TP L^{-1} respectively [6]. However, the limits of the nutrients that can induce eutrophication in tropical ecosystems have not been defined.

Algae have been used as model organisms for bioassays, especially in toxicity testing of pollutants such as ammonia [7], pesticides [8] and heavy metals [9]. As the sensitivity of algae to toxicants varies with species, it has been suggested, a battery of algal species representing all major taxonomic groups should be used in toxicity testing [10].

One of the criteria in choosing an organism for assay purposes is that it should be sensitive to low concentrations of the material [11]. One common species used in bioassays is *Pseudokirchneriella capricornutum* (formerly *Selenastrum capricornutum*) because it can be easily cultured with minimum morphological changes during the growth phase [12]. This species is also highly sensitive and can be used to assay down to 0.01 mM nitrogen [13]. There have been efforts to search for new, more sensitive test species that respond to both growth inhibitors and stimulators. For instance, in the 1980's there were attempts to use *Stigeoclonium* for bioassay of nitrogen in eutrophic waters [14]. Recently, there have been efforts to screen for suitable algae as bioassay organisms in monitoring of pollution caused by industrial wastewater in marine waters [15]. The green alga *Nephroselmis pyriformis* is used for toxicity testing of ammonia in marine waters [7] while the diatom *Nitzschia closterium* is used for ecotoxicological testing of sewage effluent [16]. Most of the algae used in bioassays are temperate species, although there have been efforts to screen tropical phytoplankton for toxicity testing of heavy metals [9].

Several algae from the University of Malaya Algae Culture Collection (UMACC) have been shown to have potential applications in bioremediation of agroindustrial wastewater and biomonitoring of heavy metals [17]. For instance, *Chlorella vulgaris* UMACC 001 was shown to be efficient in treating

rubber and palm oil mill effluent using the High Rate Algae Pond (HRAP) system [18]. Several marine algae such as *Tetraselmis*, *tetrahele* and *Chaetoceros calcitrans* are useful for toxicity testing against heavy metals [9]. The tolerance of four algae from the collection to high nitrogen levels was investigated in attempts to assess their potential use as indicator species for nitrogen enrichment [19].

The aim of this study was to assess the suitability of three tropical algae as bioassay organisms for nitrogen and phosphorus enrichment. The approach used was based on the effect of nitrogen and phosphorus enrichment on the stimulation of algal growth. This will be useful for predicting and monitoring of eutrophication in freshwater ecosystems.

MATERIALS AND METHODS

Test organisms

Three freshwater algae from the UMACC, namely *Chlorella vulgaris* UMACC 001, *Scenedesmus quadricauda* UMACC 041 and *Ankistrodesmus convolutus* UMACC 101 were used as the test organisms for the study. *Chlorella vulgaris* and *Scenedesmus quadricauda* were isolated from a fish tank at the University of Malaya farm, while *Ankistrodesmus convolutus* was isolated from Tasik Aman at Petaling Jaya [20].

Culture media

The stock cultures and inoculum were grown in Bold Basal's Medium (BBM) [21]. The Dilution Water, without nitrogen and phosphorus was used as the control [22]. As preliminary studies showed that 1% BBM supported minimal growth of the algae, it was used as the minimal medium for the study. Nitrogen ($NaNO_3$ or NH_4Cl) and phosphorus (KH_2PO_4 and K_2HPO_4) were added to this medium to investigate the effect of nutrient enrichment on algal growth. All media were buffered with 10 mM of 4-(2-hydroxyethyl)-piperazine-1-ethane-sulfonic acid (HEPES).

Experimental procedures

For all the experiments, the inoculum used was from exponential phase cultures. The inoculum was centrifuged (3000 rpm for 10 minutes), washed and re-suspended in dilution water and OD_{620} was adjusted to 0.2. The inoculum (10 mL) was added aseptically to 80 mL of minimal medium in 250 mL conical flasks, and mixed with $NaNO_3$, NH_4Cl or K_2HPO_4 and KH_2PO_4 solutions (10 mL). Ten mL inoculum and 10 mL sterile distilled water were added to the dilution water (negative control). Triplicate cultures were used for each treatment. The cultures were grown in a controlled-environment incubator with agitation (150 rpm), set at 25°C, with irradiance of 42 μmol m^{-2} s^{-1} on 12:12 h light-dark cycle (cool fluorescent light).

In the first experiment, the cultures were grown at $NaNO_3$ or NH_4Cl concentrations ranging from 0 (dilution water), 0.03, 0.15, 0.75, 3.75 to 18.75 mM. In the second experiment, the cultures were grown in minimal medium containing phosphate (KH_2PO_4 and K_2HPO_4) concentrations ranging from 0.02, 0.10, 0.50, 2.50 to 12.50 mM, with 0.03 mM of either $NaNO_3$ or NH_4Cl. The dilution water without any phosphorus and nitrogen was used as the control. All the growth experiments were conducted for 96 hours. Every 12 hours, 2 ml samples were withdrawn for cell counting using a haemacytometer (Improved Double-Neubauer).

Specific growth rate

The specific growth rate (μ, day^{-1}) was determined using the following formula:

$\mu = (\text{Ln } N_1 - \text{Ln } N_2) / (t_2 - t_1)$, where N_1 and N_2 represent the cell numbers at times t_1 and t_2 respectively within the exponential phase (96 h).

Percentage growth enhancement

The percentage growth enhancement (PGE) was calculated based on the enhancement of algal growth in medium containing nitrogen and phosphorus compared to that in dilution water. The final cell number (96 hours) was used for the calculation of PGE_{96}, as shown below:

PGE_{96} = [(Cell number in medium with nitrogen and phosphorus) - (Cell number in Dilution Water)] / (Cell number in Dilution Water) × 100%

Growth response curves based on PGE_{96} versus logarithmic concentrations of nitrogen and phosphorus were plotted. Linear regression between the two parameters was analyzed (Microsoft Excel) to obtain the regression coefficient (R^2). The effective concentration that

gave a PGE_{96} of 50% was calculated using the equation generated.

RESULTS

There was growth enhancement in response to nitrogen and phosphorus enrichment within the 96 h tests for the three algae. The endpoint parameters used to assess the suitability of the algae as test organisms for bioassay of nitrogen and phosphorus were specific growth rate (μ) and cell number attained at 96 h, which was used to derive the PGE_{96} (Tables 1 - 3; Figures 1 – 2).

Effect of $NaNO_3$ and NH_4Cl enrichment

In general, the μ of the three algae increased with increasing $NaNO_3$ and NH_4Cl concentration (Tables 1 and 2). Of the three chlorophytes, *Ankistrodesmus convolutus* attained the highest μ (0.056 and 0.100 h^{-1} for medium containing $NaNO_3$ and NH_4Cl respectively).

The response of algal growth to nitrogen enrichment, as indicated by PGE_{96} is shown in Figure 1. The correlation between PGE_{96} and $NaNO_3$ concentration was highest for *Chlorella vulgaris* (R^2 = 0.98) and lowest for *Scenedesmus quadricauda* (R^2 = 0.42). There was a strong correlation between PGE_{96} and NH_4Cl concentration (R^2 = 0.86 – 0.97) for the three algae (Figure 1b). The PGE_{96} of *Ankistrodesmus convolutus* was much higher than the other two algae when grown on NH_4Cl.

There was a wide difference in EC_{50} values (0.005 to 2.49 mM) of the three algae in response to nitrogen enrichment (Table 4). The two algae which showed the lowest EC_{50} in response to $NaNO_3$ and NH_4Cl enrichment were *Scenedesmus quadricauda* (0.0066 mM) and *Ankistrodemsus convoloutus* (0.005 mM) respectively.

Effect of phosphate enrichment

In general, there was an increase in μ (0.0053 to 0.0096 h^{-1}) of the three algae in response to phosphate enrichment. The response was similar when the algae were grown on $NaNO_3$ or NH_4Cl (Table 3).

There was a difference in PGE_{96} of the three algae in response to phosphate enrichment between the cultures grown on $NaNO_3$ and NH_4Cl (Figure 2). The regression coefficient (R^2) between PGE_{96} and phosphate concentration was higher in *Ankistrodesmus convolutus* and *Scenedesmus convolutus* than *Chlorella vulgaris* (Figure 2a). In comparison, there was a strong correlation (R^2 = 0.87 – 0.97) between the two parameters for the three algae when grown on NH_4Cl. The PGE_{96} of *Ankistrodesmus convolutus* was higher than *Scenedesmus quadricauda* and *Chlorella vulgaris* when grown on NH_4Cl (Figure 2b).

The EC_{50} of *Chlorella vulgaris* in response to phosphate enrichment was extremely high (822.7 mM) when grown on $NaNO_3$ (Table 5). In comparison, the EC_{50} of this alga was so much lower (0.12 mM) when grown on NH_4Cl. The EC_{50} values of *Ankistrodesmus convolutus* and *Scenedesmus quadricauda* were similar, and were lower when grown on NH_4Cl than on $NaNO_3$.

Table 1. Specific growth rates (μ, h^{-1}) based on the cell number of the three chlorophytes grown at different $NaNO_3$ concentrations. Data are shown as mean ± standard deviation (n = 3)

$NaNO_3$ CONCENTRATION (mM)	ALGAE		
	Chorella vulgaris	***Scendesmus quadricauda***	***Ankistrodesmus convolutus***
0	0.0047 ± 0.0007	0.0035 ± 0.0003	0.0056 ± 0.0007
0.03	0.0047 ± 0.0007	0.0055 ± 0.0002	0.0068 + 0.0007
0.15	0.0058 ± 0.0009	0.0054 ± 0.0006	0.0081 ± 0.0009
0.75	0.0063 ± 0.0006	0.0058 ± 0.0008	0.0081 ± 0.0006
3.75	0.0071 ± 0.0005	0.0062 ± 0.0006	0.0081 ± 0.0006
18.75	0.0079 ± 0.0003	0.0057 ± 0.0001	0.0086 ± 0.0006

Table 2. Specific growth rates (μ, h^{-1}) based on the cell number of the three chlorophytes grown at different NH_4Cl concentrations. Data are shown as mean ± standard deviation (n = 3)

NH_4Cl CONCENTRATION (mM) .	ALGAE		
	Chorella vulgaris	***Scendesmus quadricauda***	***Ankistrodesmus convolutus***
0	0.0058 ± 0.0005	0.0044 ± 0.0001	0.0060 ± 0.0007
0.03	0.0066 ± 0.0002	0.0046 ± 0.0002	0.0082 ± 0.0002
0.15	0.0075 ± 0.0003	0.0050 ± 0.0005	0.0090 ± 0.0008
0.75	0.0076 ± 0.0002	0.0056 ± 0.0003	0.0096 ± 0.0002
3.75	0.0081 ± 0.0003	0.0065 ± 0.0002	0.0094 ± 0.0002
18.75	0.0082 ± 0.0003	0.0068 ± 0.0002	0.0100 ± 0.0001

Table 3. Specific growth rates (μ, h^{-1}) based on the cell number of the three chlorophytes grown at different phosphate concentrations in the presence of 0.03 mM $NaNO_3$ or NH_4Cl. Data are shown as mean ± standard deviation (n = 3)

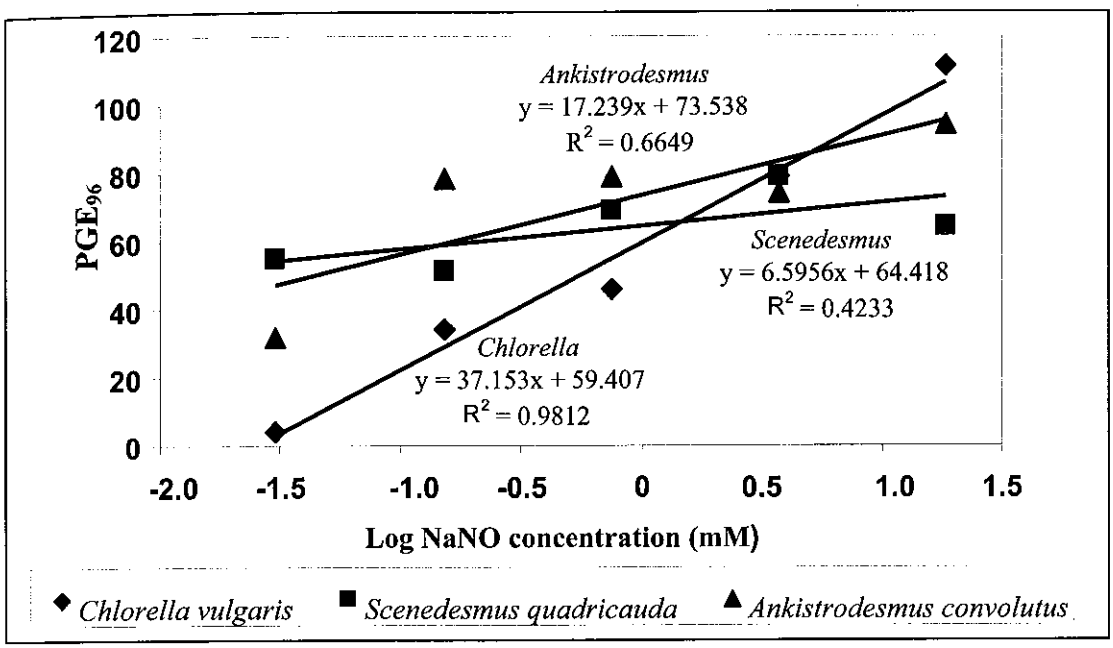
PHOSPHATE CONCENTRATION (mM)	ALGAE					
	Chorella vulgaris		*Scendesmus quadricauda*		*Ankistrodesmus convolutus*	
	$NaNO_3$	NH_4Cl	$NaNO_3$	NH_4Cl	$NaNO_3$	NH_4Cl
0	0.0074 ± 0.0002	0.0059 ± 0.0001	0.0057 ± 0.0006	0.0058 ± 0.0002	0.0060 ± 0.0005	0.0053 ± 0.0002
0.02	0.0078 ± 0.0004	0.0076 ± 0.0001	0.0070 ± 0.0003	0.0074 ± 0.0001	0.0066 ± 0.0004	0.0081 ± 0.0004
0.10	0.0079 ± 0.0007	0.0076 ± 0.0001	0.0070 ± 0.0005	0.0074 ± 0.0003	0.0077 ± 0.0003	0.0087 ± 0.0005
0.50	0.0079 ± 0.0007	0.0076 ± 0.0001	0.0078 ± 0.0005	0.0077 ± 0.0004	0.0079 ± 0.0003	0.0087 ± 0.0003
2.50	0.0080 ± 0.0001	0.0081 ± 0.0001	0.0079 ± 0.0001	0.0079 ± 0.0004	0.0096 ± 0.0008	0.0091 ± 0.0005
12.5	0.0085 ± 0.0004	0.0084 ± 0.0001	0.0080 ± 0.0008	0.0082 ± 0.0002	0.0096 ± 0.0002	0.0092 ± 0.0005

Table 4. Effective concentrations of $NaNO_3$ and NH_4Cl that resulted in PGE_{96} of 50% (EC_{50})

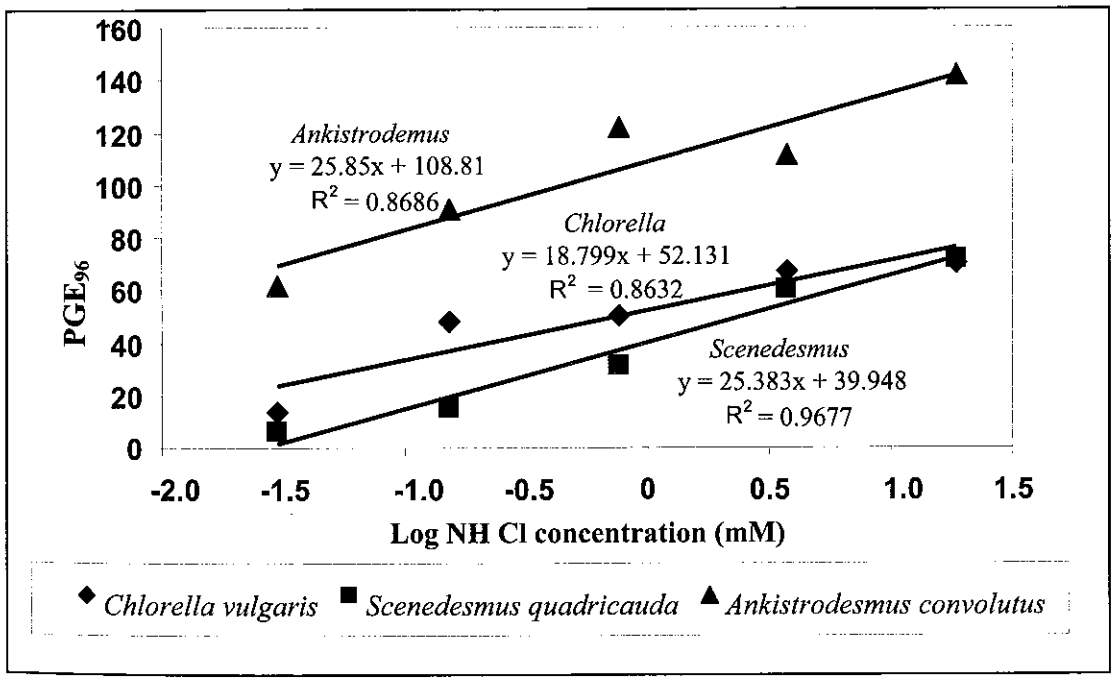
ALGAE	$NaNO_3$ (mM)	NH_4Cl (mM)
Chorella vulgaris	0.56	0.77
Scendesmus quadricauda	0.0066	2.49
Ankistrodesmus convolutus	0.040	0.005

Table 5. Effective concentrations of phosphate that resulted in PGE_{96} of 50% (EC_{50}) for the algae grown in medium containing $NaNO_3$ or NH_4Cl (0.03 mM)

ALGAE	NITROGEN SOURCE	
	$NaNO_3$	NH_4Cl
Chorella vulgaris	822.70	0.12
Scendesmus quadricauda	0.20	0.08
Ankistrodesmus convolutus	0.22	0.08

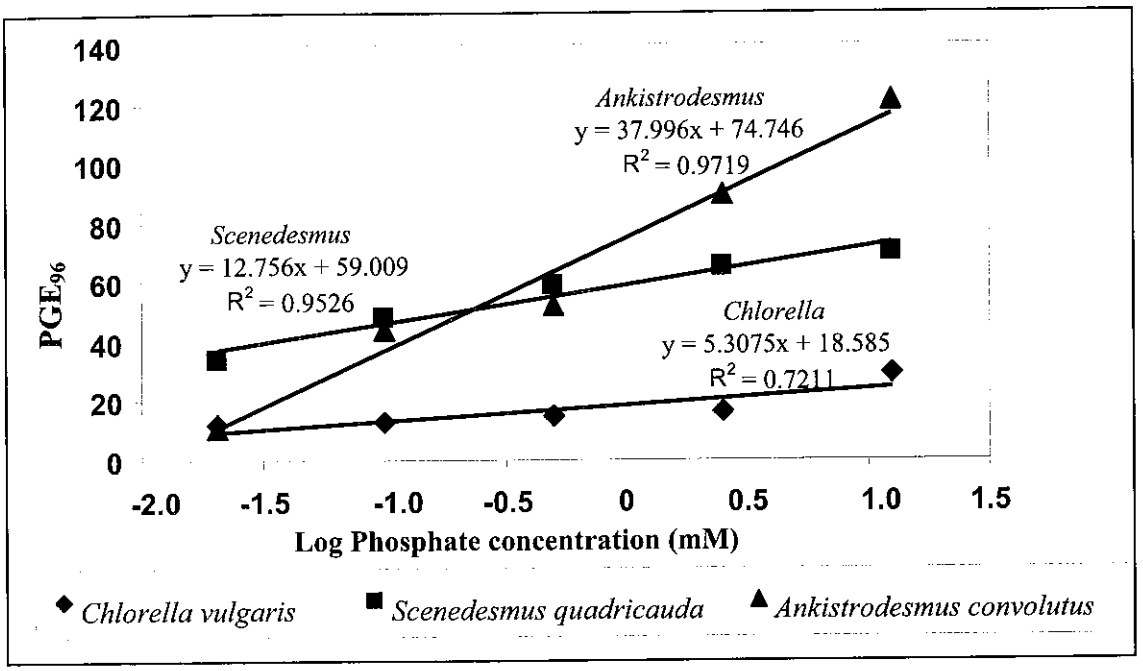


a) $NaNO_3$

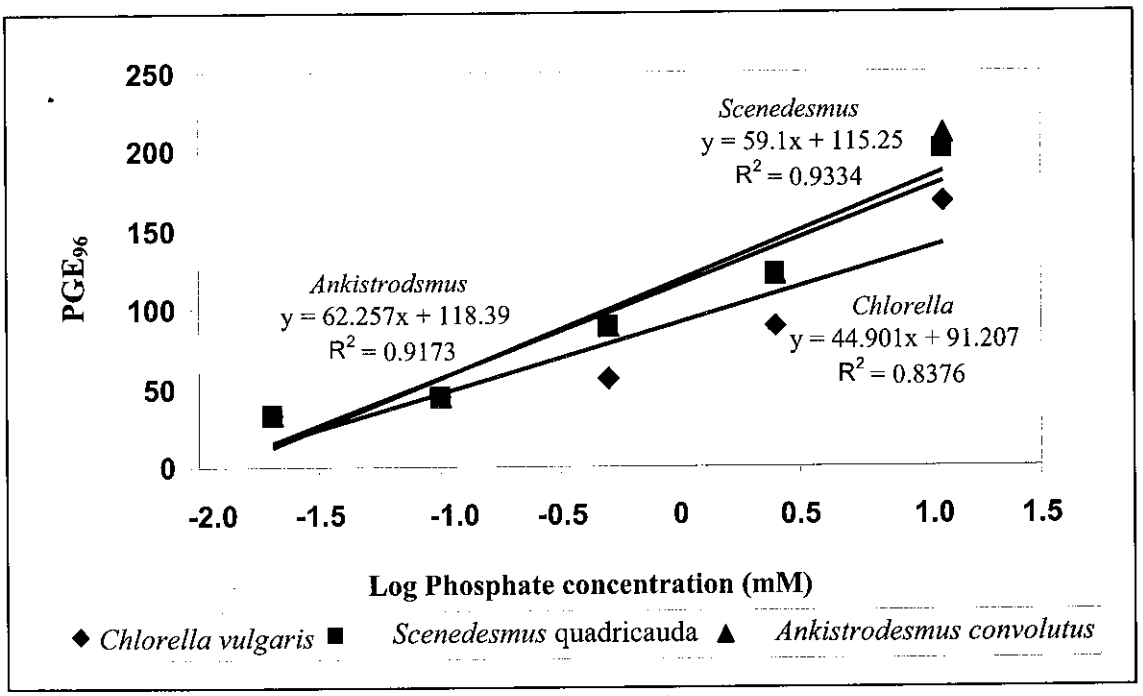


b) NH_4Cl

Figures 1a and 1b. Dose-response relationship between PGE_{96} and nitrogen concentration of the three algae. The equations and R^2 values shown are from linear regression analysis between the two parameters. PGE_{96} = Percentage Growth Enhancement based on cell number attained at 96 h compared to that attained by the cultures grown in Dilution Water. The cultures were grown in minimal medium added with increasing concentration of a) $NaNO_3$ and b) NH_4Cl. Each point represents the mean value of three replicate cultures.



a) Grown on $NaNO_3$



b) Grown on NH_4Cl

Figures 2a and 2b. Dose-response relationship between PGE_{96} and phosphate concentration of the three algae. The equations and R^2 values shown are from linear regression analysis between the two parameters. PGE_{96} = Percentage Growth Enhancement based on cell number attained at 96 h compared to that attained by the cultures grown in Dilution Water. The cultures were grown in minimal medium added with increasing concentration of phosphate, containing either 0.03 mM of a) $NaNO_3$ or b) NH_4Cl. Each point represents the mean value of three replicate cultures.

DISCUSSION

The present study is the first that aimed to assess the suitability of tropical algae as test organisms for the bioassay of nitrogen and phosphorus enrichment. The approach used was based on the stimulatory effect on algal growth in response to the addition of nutrients in the minimal medium. The minimal medium (1% BBM) containing 0.03 mM nitrogen and 0.02 mM phosphorus was suitable for the bioassay of nitrogen and phosphorus, as the amounts of nutrients supported minimal growth of the algae. The approach used was different from other bioassays, which focus on the inhibitory effect of the test materials on algal growth [7, 16]. Short growth duration (96 h) was used for the bioassay.

The nitrogen and phosphorus concentrations used in the tests ranged from 0.03 to 18.75 mM and 0.02 to 12.50 mM respectively. This differs from our previous study where the algae were grown for 8 days at high concentrations of $NaNO_3$ and NH_4Cl, ranging from 2.9 to 250 mM [19]. The levels used here are relevant to that present in the environment. For example, clean freshwater contains about 0.07 mM $NaNO_3$ [23] while agro-industrial wastewaters such as rubber effluent may contain 5.6 – 70.0 mM NH_3-N and 0.35 mM NO_3 [18]. The limit of total nitrogen that induces eutrophication in lakes is 0.09 mM [6]. In freshwater lakes, the phosphorus concentrations may range from 0.0002 to 0.0080 mM [24] while in rubber effluent the range is from 0.16 to 1.52 mM [18].

Bioassays using algae can be based on the effects of the test materials on growth [16], cell motility [15], photosynthesis [25] and dissolved oxygen production [8]. In terms of the effect on growth, it can be based on cell yield (difference between final and initial cell number) [16] or μ [7]. In the present study, the percentage growth enhancement based on cell number attained at 96 h (PGE_{96}) appeared to be a better parameter than μ for the bioassay of nitrogen and phosphorus. The percentage enhancement of μ due to nitrogen and phosphorus enrichment ranged from 14.9 – 73.6%, while for PGE_{96}, it may reach up to 140%.

In general, testing of natural waters is usually based on IC_{50} or Algal Growth Potential (AGP). The first parameter is more useful for toxicity testing rather than assessing growth stimulation due to nutrient enrichment [7]. The AGP test is based on the principle that the maximum cell yield or biomass attained by the test alga is proportional to amount of nutrient present in the water sample [26]. For example, the AGP assay based on *Pseudokirchneriella capricornutum* is used to detect the phosphorus level in agricultural runoff [26]. The water samples will first be sterilized by gamma-radiation before being inoculated with the test organism and the algal assay takes 9 –12 days under standard growth conditions. Thus, longer time is required for AGP test compared to bioassay based on PGE_{96} employed in this study.

The three algae could grow even at NH_4Cl concentration as high as 18.75 mM. This finding contrasted with other studies which showed that high levels of ammonium and ammonia are toxic to algae. For example, NH_4^+ and NH_3 levels at above 30 μM can inhibit growth of *Nephroselmis pyriformia* [7]. Algae which are sensitive to high ammonium concentrations are usually those inhabit environments with constant low levels of the nitrogen source. The species used by Kallqvist and Svenson [7] is a marine chlorophyte, and most probably isolated from marine waters with low ammonium level. In comparison, the three chlorophytes used in the present study were isolated from nitrogen-rich habitats. For example, *Chlorella vulgaris* UMACC 001 was isolated from a fish tank at an experimental farm [20].

One of the important criteria for the selection of algae for bioassays is it should show high sensitivity in its response to the toxicant or stimulant. It should also show a distinct dose-response relationship with regards to the testing material. Of the three algae, only the PGE_{96} of *Chlorella vulgaris* showed a significant linear relationship ($R^2 = 0.98$) with $NaNO_3$ concentration. Thus, this alga would be a suitable organism for the bioassay of $NaNO_3$. The response of PGE_{96} to NH_4Cl enrichment showed a strong linear relationship ($R^2 = 0.86 - 0.97$) for the three algae. However, *Ankistrodesmus convolutus* was most sensitive to the stimulation of NH_4Cl, with EC_{50} of only 0.005 mM. Thus, this alga would be a useful test organism for the bioassay of ammonium. Our previous study showed that *Chlorella vulgaris* is

most tolerant to high nitrogen levels, being able to grow even at 250 mM $NaNO_3$ or NH_4Cl [9]. In comparison, *Ankistrodesmus convolutus* is most sensitive, as its growth is inhibited at nitrogen levels above 75 mM. Thus, a highly tolerant species like *Chlorella vulgaris* will be useful for treating nitrogen-rich wastewater while sensitive species like *Ankistrodesmus convolutus* will be useful as test organism for bioassay.

The dose-response between PGE_{96} and phosphate enrichment of the three algae showed strong linear relationship (R^2 = 0.72 – 0.97) However, the sensitivity of the algae was dependent on whether they were grown on $NaNO_3$ or NH_4Cl. When grown on $NaNO_3$, there was hardly any stimulation of growth of *Chlorella vulgaris* in response to phosphate enrichment (EC_{50} = 822.7 mM). Thus, *Scenedesmus quadricauda* and *Ankistrodesmus convolutus* (not *Chlorella vulgaris*) would be suitable for the bioassay of phosphate in water sample rich in $NaNO_3$. However, the three algae would be useful as test organisms for the bioassay of phosphate in water sample containing NH_4Cl as the dominant nitrogen component.

CONCLUSION

The PGE_{96} was a suitable parameter for the bioassay of nitrogen and phosphorus using algae. Of the three algae, *Chlorella vulgaris* was a potential test organism for the bioassay of $NaNO_3$ while *Ankistrodesmus convolutus* was suitable for NH_4Cl bioassay. All the three algae were suitable test organisms for the bioassay of phosphate when grown on NH_4Cl. However, *Ankistrodesmus convolutus* and *Scenedesmus quadricauda* were more suitable than *Chlorella vulgaris* for the bioassay of phosphate, when grown on $NaNO_3$. Further studies to assess the potential use of the three algae as bioassay organisms for the testing of natural lake water and wastewater samples should be conducted.

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