

## **APPENDICES**

Appendix 1 : List of facilities and equipment used

Facilities :

sterile inoculation chamber  
adequate supply of running tap water and, deionised and distilled water  
incubation chamber with ample space  
sample storage (refrigerator)

Equipment :

refractometer  
pH meter  
thermometer  
calibrated light meter  
balances  
glasswares (beakers, volumetric flasks, test tubes, erlenmeyer flasks)  
24-welled Nunclon multiwell plates  
calibrated pipettors and disposable pipette tips  
magnetic stirrers  
pasteur pipettes  
HDPE bottles and tubes  
vacuum filtration apparatus  
membrane filters; 0.45 µm pore size  
autoclave  
microscope  
Neubauer brightline haemocytometer  
UV-VIS Spectrophotometer  
Multiskan MCC/340 MKII  
ovens  
separatory funnels  
Inductively-Coupled Plasma - Atomic Emission Spectrophotometer  
(ICP-AES)

## Appendix 2 : Composition of the Conway media (AQUACOP, 1984)

Stock Solution	Constituent	Amount
A. Main mineral solution	<ul style="list-style-type: none"> <li>• Sodium nitrate, NaNO<sub>3</sub> or Potassium nitrate, KNO<sub>3</sub></li> <li>• Disodium EDTA C<sub>10</sub>H<sub>12</sub>N<sub>2</sub>Na<sub>2</sub>O<sub>8</sub>.2H<sub>2</sub>O</li> <li>• Boric acid, H<sub>3</sub>BO<sub>3</sub></li> <li>• Sodiumdihydrogen phosphate, NaH<sub>2</sub>PO<sub>4</sub>.4H<sub>2</sub>O</li> <li>• Ferric choride, FeCl<sub>3</sub>.6H<sub>2</sub>O</li> <li>• Manganese chloride MnCl<sub>2</sub>.4H<sub>2</sub>O</li> <li>• Trace metal solution</li> <li>• Distilled water</li> </ul>	<ul style="list-style-type: none"> <li>100g</li> <li>116g</li> <li>45g</li> <li>33.6g</li> <li>20g</li> <li>1.30g</li> <li>0.36g</li> <li>1mL</li> <li>1000mL</li> </ul>
B. Trace metal solution	<ul style="list-style-type: none"> <li>• Zinc chloride, ZnCl<sub>2</sub></li> <li>• Cobalt chloride, CoCl<sub>2</sub>.6H<sub>2</sub>O</li> <li>• Ammonium molybdate (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>.4H<sub>2</sub>O</li> <li>• Copper sulphate, CuSO<sub>4</sub>.5H<sub>2</sub>O</li> <li>• Distilled H<sub>2</sub>O</li> </ul>	<ul style="list-style-type: none"> <li>2.10g</li> <li>2.00g</li> <li>0.90g</li> <li>2.00g</li> <li>100mL</li> </ul>
C. Vitamin solution	<ul style="list-style-type: none"> <li>• Thiamine chlorhydrate (vitamin B1)</li> <li>• Cyanocobalamine (vitamin B12)</li> <li>• Distilled water</li> </ul>	<ul style="list-style-type: none"> <li>200mg</li> <li>10mg</li> <li>100mL</li> </ul>
D. Silicate solution	<ul style="list-style-type: none"> <li>• Sodium silicate, Na<sub>2</sub>SiO<sub>3</sub></li> <li>• Distilled water</li> </ul>	<ul style="list-style-type: none"> <li>20g</li> <li>1000mL</li> </ul>
E. Nitrate solution	<ul style="list-style-type: none"> <li>• Potassium nitrate, KNO<sub>3</sub></li> <li>• Distilled water</li> </ul>	<ul style="list-style-type: none"> <li>100g</li> <li>1000mL</li> </ul>

Appendix 3 : Composition of commercial sea salt MARINE ENVIRONMENT  
Dual Phase Formula.

Element	Amount (mgL <sup>-1</sup> )
Chloride	20,000
Sodium	10,599
Sulfate	2,506
Mâgnesium	1,360
Calcium	440
Potassium	393
Bromine	55
Strontium	7.2
Phosphate	0.01
Iodide	0.12
Aluminium	0.02
Iron	0.12
Zinc	0.045
Nickel	0.0023
Copper	0.03
Lead	0.004
Thallium	0.0001
Cadmium	0.0003
Selenium	0.005
Vanadium	0.0003

Note : Information was provided by the manufacturer AQUA CRAFT™ INC., California, from the analyses done by the University of Missouri, USA.

Appendix 4(1) : Solvent extraction technique for Cd and Cu from seawater  
(Kenneth and Roberts, 1979)

125 mL seawater sample put into a 250mL conical flask

↓ (one or two seawater sample spiked for known concentration of metal  
↓ for standard addition test)

acidified to pH4 with nitric acid

↓ transferred into a 250mL separatory funnel  
+ 1mL ammonium acetate  
+0.5 mL 1% (w/v) APDC-DDDC solution  
+4 mL Q-chloroform

↓ Mixture shaken vigorously for 2 minutes

↓ Left for 5 minutes for phase separation

↓ Separated organic fraction drained into 125 mL separatory funnel  
+ 2 mL 7.5 M Q-nitric acid

↓ 3 mL Q-chloroform added to original seawater sample for second extraction

↓ second separated fraction was combined with the first in the 125 mL separatory funnel

↓ shaken vigorously for 2 minutes

↓ left for 5 minutes to allow separation again

↓ the organic phase discarded

↓ the acid phase (containing the back-extracted metal) drained into 10 mL polyethylene vial

↓ separatory funnel rinsed with 1mL Q-nitric acid and combined with the acid fraction

↓ final volume of 3 mL stored in 10 mL polyethylene vial

↓ a 41.7 fold preconcentration of sample obtained

Appendix 4(2) : Solvent extraction technique for Mn from seawater (Grasshoff, 1983).

50mL of filtered seawater in 250mL separatory funnel

↓  
(one or two seawater sample spiked for known concentration of metal for  
standard addition test)

pH adjusted to 9-9.5 with ammonium hydroxide solution

↓  
5 mL oxine reagent added

↓  
mixture shaken for 4 minutes

↓  
left for 10 minutes to allow phase separation

↓  
lower organic layer drained into 125 mL separatory funnel

↓  
5 mL Q-chloroform added to extracted seawater for second extraction

↓  
shaken for 4 minutes

↓  
left for 10 minutes to allow separation again

↓  
both organic extracts combined in 125 mL separatory funnel  
+ 2 mL 7 M ultrapure nitric acid

↓  
mixture shaken for 2 minutes

↓  
after separation, lower organic phase discarded

↓  
2 mL aqueous phase (containing the back-extracted Mn) drained into 10 mL  
polyethylene vial

↓  
separatory funnel rinsed with 2 X 0.5 mL, 7 M ultrapure nitric acid

↓  
the rinse + the extracts = final volume of 3 mL stored in 10 mL polyethylene vial

↓  
a 16.7 fold preconcentration of sample obtained.

**Appendix 4(3) : Standard addition test for seawater analyses**

**(a) Preparation of single standard for spiking**

Single standards ( $5 \mu\text{g mL}^{-1}$ ) for each metal were prepared from the  $1000 \text{ mg L}^{-1}$  stock standard solution (MERCK).

**(b) Spiking, solvent extraction and calculation**

0.1mL of the (single) standard was spiked into the seawater sample



solvent extraction procedure was carried out as in Appendix 4 (1) and (2)

**Calculation (Example) :**

0.1 mL of single standard contains  $5 \mu\text{g mL}^{-1}$  Cu

100 mL of seawater sample resulted in dilution to  $5 \times 0.1/100 = 0.005 \mu\text{g mL}^{-1}$  Cu

Therefore in the final volume of 3 mL it is concentrated to =

$0.005 \times 100/3 = 0.167 \mu\text{g mL}^{-1}$  Cu (expected value)

**(d) Calculation of heavy metal extraction recovery efficiency**

(i) Recovery efficiency (%) = tabulated value of the spiked concentration X 100  
expected value of the spiked concentration

(ii) Efficiency of trace metal solvent extraction technique for seawater using the standard addition test :

Element	Recovery efficiency (%)
Cd	89.8-101.8%
Cu	83.8-95.8%
Mn	89.8-95.8%

### Appendix 5(1) : Using the TOXSTAT (Release 3.2) software programme (Gulley *et al.*, 1990)

(a) After the TOXSTAT programme had been accessed, any key was pressed to view the MAIN MENU. Choices in the MAIN MENU were accessed by using <F> function keys.

#### (b) Data Input :

After pressing <F4> key for 'Data Input' and <O> to open a new file, The 'Titles' and 'Values' screens were presented. Data were entered using the arrow keys to move to an appropriate data cell and pressing <ENTER>. On the 'Titles' screen, the title of the analysis, the number of groups (treatments) including the control, group ID (the series of concentration including the control) with units, and the number of replicates in a each group were entered. Actual initial heavy metal concentrations as determined by ICP-AES were used if available. Otherwise the nominal concentrations were used.

The 'Values' screen was viewed by pressing <F1>. Each group (treatment) had its own value screen indicating the group ID and number of replicates. The individual data values (cell counts) for each group were entered at the right value screen. The <F5> and <F6> keys were used to move to the previous or next group.

After data entry had been completed the <F8> key was pressed. <W> was chosen to write and save the data to a file.

#### (c) Listing data and summary statistics :

After pressing the <F3> key at the MAIN MENU, the arrow keys were used to move between the fields, and the space bar to choose 'YES' for 'List Data' and 'Summary Statistics' and 'PRINTER' for the output. The <F5> key was used to execute the task.

#### (d) Data transformation and test of assumptions :

The <F2> key at the MAIN MENU was used to access the 'Data Transformation' menu. The arrow keys were used to move between the fields and the space bar to change the choices in each field. Cell counts from the phytoplankton were transformed using a 'log10 transformation'.

The Shapiro-Wilk's test for normality was chosen as there were <50 values in the data set followed by the Bartlett's test for homogeneity of variance. Then 'PRINTER' was chosen for the output and <F5> to run the tests. The printouts indicated whether the data passed the tests for normality and homogeneity of variance.

#### (e) Statistical analyses :

The <F3> key at the MAIN MENU was selected to do statistical tests. If the data passed both the normality and the homogeneity of variance tests, the parametric statistics were used. If the test had equal replicates, the AVOVA and Dunnett's tests were used to analyse the toxicity test data. If in the event of unequal replicates, the ANOVA and Bonferroni's tests were done.

If the data failed one or both of tests, non-parametric tests were used. The Steel's Many-One Rank test was used if the tests had equal replicates while the Wilcoxon Rank Sum test was done if the replicates were unequal.

The arrow keys were used to select between the field while the space bar to select the choices and to send the output to the printer.

As the data is such that an adverse treatment response would be a smaller number than the control response (i.e. lower cell number) the arrow keys and space bar was used to choose "<" in H<sub>0</sub>.

<F5> was chosen to execute the statistical analysis.

## Appendix 5(2)

Appendix 5(2) : Output from statistical analyses by TOXSTAT programme

(a) List of data and summary of statistics

TITLE: C.calcitrans - Cadmium (with EDTA)/A1.1  
 FILE: allR5  
 TRANSFORM: NO TRANSFORM                    NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	0	1	269.0000	269.0000
1	0	2	265.5000	265.5000
1	0	3	295.5000	295.5000
2	5.8	1	215.2000	215.2000
2	5.8	2	208.2000	208.2000
2	5.8	3	222.1000	222.1000
3	11.8	1	151.0000	151.0000
3	11.8	2	128.4000	128.4000
3	11.8	3	152.7000	152.7000
4	23.7	1	5.2000	5.2000
4	23.7	2	7.0000	7.0000
4	23.7	3	3.5000	3.5000
5	47.7	1	5.2000	5.2000
5	47.7	2	3.5000	3.5000
5	47.7	3	7.0000	7.0000
6	97.4	1	5.2000	5.2000
6	97.4	2	5.2000	5.2000
6	97.4	3	3.5000	3.5000

C.calcitrans - Cadmium (with EDTA)/A1.1  
 File: allR5                                    Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA    TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	0	3	265.500	295.500	276.667
2	5.8	3	208.200	222.100	215.167
3	11.8	3	128.400	152.700	144.033
4	23.7	3	3.500	7.000	5.233
5	47.7	3	3.500	7.000	5.233
6	97.4	3	3.500	5.200	4.633

C.calcitrans - Cadmium (with EDTA)/A1.1  
 File: allR5                                    Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA    TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	0	269.083	16.404	9.471
2	5.8	48.303	6.950	4.013
3	11.8	184.023	13.566	7.832
4	23.7	3.063	1.750	1.011
5	47.7	3.063	1.750	1.011
6	97.4	0.963	0.981	0.567

continued...

(b) Results of tests for normality and homogeneity of variance

C.calcitrans - Cadmium (with EDTA)/A1.1  
File: a11R5      Transform: LOG BASE 10(Y)

Shapiro Wilks test for normality

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D = 0.116

W = 0.916

Critical W (P = 0.05) (n = 18) = 0.897  
Critical W (P = 0.01) (n = 18) = 0.858

---

Data PASS normality test at P=0.01 level. Continue analysis.

C.calcitrans - Cadmium (with EDTA)/A1.1  
File: a11R5      Transform: LOG BASE 10(Y)

Bartletts test for homogeneity of variance

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Calculated B statistic = 11.00  
Table Chi-square value = 15.09 (alpha = 0.01)  
Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 2.00  
Used for Chi-square table value ==> df (#groups-1) = 5

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Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replica e size is used to calculate the B statistic (see above).

continued...

## (c) Results of statistical analyses

C.calcitrans - Cadmium (with EDTA)/A1.1  
 File: a11R5                          Transform: LOG BASE 10(Y)

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	11.981	2.396	239.600
Within (Error)	12	0.116	0.010	
Total	17	12.097		

Critical F value = 3.11 (0.05,5,12)  
 Since F > Critical F REJECT Ho:All groups equal

C.calcitrans - Cadmium (with EDTA)/A1.1  
 File: a11R5                          Transform: LOG BASE 10(Y)

DUNNETTS TEST - TABLE 1 OF 2

Ho:Control&lt;Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	0	2.441	276.667		
2	5.8	2.333	215.167	1.333	
3	11.8	2.157	144.033	3.482 *	
4	23.7	0.702	5.233	21.307 *	
5	47.7	0.702	5.233	21.307 *	
6	97.4	0.659	4.633	21.834 *	

Dunnett table value = 2.50                  (1 Tailed Value, P=0.05, df=12,5)

C.calcitrans - Cadmium (with EDTA)/A1.1  
 File: a11R5                          Transform: LOG BASE 10(Y)

DUNNETTS TEST - TABLE 2 OF 2

Ho:Control&lt;Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	0	3	103.633	37.5	61.500
2	5.8	3	103.633	37.5	132.633
3	11.8	3	103.633	37.5	271.433
4	23.7	3	103.633	37.5	271.433
5	47.7	3	103.633	37.5	272.033
6	97.4	3	103.633	37.5	

Appendix 6(1) : Using the ICPIN programme (Norberg-King, 1993)

The programme was accessed by typing "ICPIN" at the DOS prompt and hitting <ENTER>.

(a) Data input

Data Entry/Edit screen was displayed by pressing any key at the ICp startup screen. The arrow keys were used to move between columns and fields, and <BACKSPACE> to change a value. The concentrations tested and the organism responses were entered at the Data Entry/Edit screen.

After data entry had been completed, the command OPTIONS, was selected and the information identifying the data (test start date, test end date, chemical, test species, test duration, test type, water type, concentration units, and user name) was entered. At the OPTIONS screen also, the "P" value (25 or 50) was entered while 'PRINTER' was selected using the spacebar for output of the data and ICp values.

After entering the data and selecting the OPTIONS, the command RUN was selected to execute the programme.

QUIT was selected from the command menu to exit the programme.

(b) Interpreting the Output

The ICp value was shown as "The Linear Interpolation Estimate" while the chosen "P" value as "Entered P Value". As there were  $\leq 6$  replicates per treatment, the "Expanded Confidence Limits" were reported.

## Appendix 6(2)

### Appendix 6 (2) : Output from analyses by ICPIN programme

#### (a) IC<sub>25</sub> value

Conc.	ID	1	2	3	4	5	6
Conc.	Tested	0	5.8	11.8	23.7	47.7	97.4
Response	1	269.0	215.2	151.0	5.2	5.2	5.2
Response	2	265.5	208.2	128.4	7.0	3.5	5.2
Response	3	265.5	222.1	152.7	3.5	7.0	3.5

\*\*\* Inhibition Concentration Percentage Estimate \*\*\*

Toxicant/Effluent: cadmium (with EDTA)

Test Start Date: 15/12 Test Ending Date: 19/12/95

Test Species: Chaetoceros calcitrans

Test Duration: 96h

DATA FILE: a11R5.icp

Conc.	Number	Concentration	Response	Std.	Pooled
ID	Replicates	ppm	Means	Dev.	Response Means
1	3	0.000	266.667	2.021	266.667
2	3	5.800	215.167	6.950	215.167
3	3	11.800	144.033	13.566	144.033
4	3	23.700	5.233	1.750	5.233
5	3	47.700	5.233	1.750	5.233
6	3	97.400	4.633	0.981	4.633

The Linear Interpolation Estimate: 7.0793 Entered P Value: 25

Number of Resamplings: 80

The Bootstrap Estimates Mean: 7.0931 Standard Deviation: 0.2675

Original Confidence Limits: Lower: 6.5695 Upper: 7.6180

Expanded Confidence Limits: Lower: 6.0087 Upper: 8.2106

Resampling time in Seconds: 1.42 Random\_Seed: 1393306968

#### (b) IC<sub>50</sub> value

Conc.	ID	1	2	3	4	5	6
Conc.	Tested	0	5.8	11.8	23.7	47.7	97.4
Response	1	269.0	215.2	151.0	5.2	5.2	5.2
Response	2	265.5	208.2	128.4	7.0	3.5	5.2
Response	3	265.5	222.1	152.7	3.5	7.0	3.5

\*\*\* Inhibition Concentration Percentage Estimate \*\*\*

Toxicant/Effluent: cadmium (with EDTA)

Test Start Date: 15/12 Test Ending Date: 19/12/95

Test Species: Chaetoceros calcitrans

Test Duration: 96h

DATA FILE: a11R5.icp

Conc.	Number	Concentration	Response	Std.	Pooled
ID	Replicates	ppm	Means	Dev.	Response Means
1	3	0.000	266.667	2.021	266.667
2	3	5.800	215.167	6.950	215.167
3	3	11.800	144.033	13.566	144.033
4	3	23.700	5.233	1.750	5.233
5	3	47.700	5.233	1.750	5.233
6	3	97.400	4.633	0.981	4.633

The Linear Interpolation Estimate: 12.7174 Entered P Value: 50

Number of Resamplings: 80

The Bootstrap Estimates Mean: 12.7114 Standard Deviation: 0.5579

Original Confidence Limits: Lower: 11.4185 Upper: 13.3628

Expanded Confidence Limits: Lower: 9.9898 Upper: 14.0728

Resampling time in Seconds: 1.42 Random Seed: 1456015512

## Appendix 7

Appendix 7 : Raw data of experiment to determine correlation between OD<sub>620nm</sub> (measured by the Multiskan and Spectrophotometer) and cell counts

(1) *Chaetoceros calcitrans*

Culture dilution (factor=0.5)	OD <sub>620nm</sub>		Average cell count (X 10 <sup>4</sup> cells mL <sup>-1</sup> )
	Multiskan	Spectrophotometer	
0 X	0.179	0.374	300.3 ± 18.4
2 X	0.086	0.190	152.1 ± 13.2
4 X	0.045	0.099	71.3 ± 6.0
8 X	0.030	0.055	37.2 ± 7.6
16 X	0.016	0.032	18.3 ± 5.3
32 X	~ 0.010	0.021	11.4 ± 3.3
64 X	0.006	0.018	4.3 ± 1.1
128 X	0.004	0.008	2.0 ± 0.7

(2) *Isochrysis galbana*

Culture dilution (factor=0.5)	OD <sub>620nm</sub>		Average cell count (X 10 <sup>4</sup> cells mL <sup>-1</sup> )
	Multiskan	Spectrophotometer	
0 X	0.158	0.358	607.9 ± 21.6
2 X	0.082	0.188	304.4 ± 13.2
4 X	0.041	0.101	150.3 ± 12.3
8 X	0.026	0.056	72.4 ± 7.8
16 X	0.014	0.034	33.7 ± 5.2
32 X	0.010	0.023	19.4 ± 2.4
64 X	0.007	0.015	10.9 ± 2.5

(3) *Tetraselmis tetrahele*

Culture dilution (factor=0.5)	OD <sub>620nm</sub>		Average cell count (X 10 <sup>4</sup> cells mL <sup>-1</sup> )
	Multiskan	Spectrophotometer	
0 X	0.135	0.272	84.2 ± 7.4
2 X	0.071	0.132	41.2 ± 6.9
4 X	0.040	0.069	21.3 ± 2.5
8 X	0.022	0.035	10.9 ± 1.7
16 X	0.014	0.020	5.5 ± 1.4
32 X	0.010	0.014	3.1 ± 0.9

(4) *Tetraselmis* sp.

Culture dilution (factor=0.5)	OD <sub>620nm</sub>		Average cell count (X 10 <sup>4</sup> cells mL <sup>-1</sup> )
	Multiskan	Spectrophotometer	
0 X	0.278	0.536	204.5 ± 8.1
2 X	0.143	0.274	97.2 ± 7.2
4 X	0.077	0.141	45.5 ± 9.9
8 X	0.044	0.078	23.0 ± 3.6
16 X	0.026	0.043	12.3 ± 1.6
32 X	0.015	0.027	5.9 ± 1.4

## Appendix 8(1)

Appendix 8 (1): Raw data of cadmium range-finding tests with four selected marine phytoplankton, in the presence of EDTA

a) *C. calcitrans*

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	0.01	0.1	1.0	10.0	100.0
A1	Initial pH	7.9	7.9	8.0	8.0	8.0	8.0
	96h OD $\pm$ SD (n=3) <sup>a</sup>	0.120 $\pm$ 0.010	0.111 $\pm$ 0.005	0.102 $\pm$ 0.002	0.099 $\pm$ 0.003	0.090 $\pm$ 0.010	0.008 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	201.3 $\pm$ 17.2	185.7 $\pm$ 7.9	170.1 $\pm$ 3.5	164.9 $\pm$ 4.6	149.3 $\pm$ 17.4	7.0 $\pm$ 1.2
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	208.0 $\pm$ 5.7	187.0 $\pm$ 6.2	175.0 $\pm$ 9.6	162.0 $\pm$ 6.8	154.0 $\pm$ 4.0	5.3 $\pm$ 0.5

b) *I. galbana*

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	0.01	0.1	1.0	10.0	100.0
A2	Initial pH	7.9	8.0	8.1	8.0	8.1	8.1
	96h OD $\pm$ SD (n=3) <sup>a</sup>	0.058 $\pm$ 0.002	0.056 $\pm$ 0.002	0.055 $\pm$ 0.002	0.053 $\pm$ 0.001	0.019 $\pm$ 0.002	0.006 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	209.4 $\pm$ 8.0	201.4 $\pm$ 8.0	197.4 $\pm$ 6.9	189.4 $\pm$ 4.0	53.8 $\pm$ 6.9	3.2 $\pm$ 2.3
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	205.0 $\pm$ 2.2	205.0 $\pm$ 4.7	203.0 $\pm$ 5.1	177.0 $\pm$ 14.0	51.0 $\pm$ 4.1	6.0 $\pm$ 0.8

c) *T. tetrahele*

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	0.01	0.1	1.0	10.0	100.0
A3	Initial pH	8.0	8.0	8.0	8.0	8.0	8.0
	96h OD $\pm$ SD (n=3) <sup>a</sup>	0.072 $\pm$ 0.001	0.070 $\pm$ 0.001	0.068 $\pm$ 0.002	0.066 $\pm$ 0.004	0.057 $\pm$ 0.001	0.007 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	42.8 $\pm$ 0.7	41.5 $\pm$ 0.7	40.2 $\pm$ 1.1	38.9 $\pm$ 2.4	33.1 $\pm$ 0.6	0.9 $\pm$ 0.4
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	41.0 $\pm$ 3.4	42.0 $\pm$ 6.2	39.0 $\pm$ 6.0	37.0 $\pm$ 1.4	34.0 $\pm$ 2.2	1.3 $\pm$ 0.5

d) *T. sp.*

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	0.01	0.1	1.0	10.0	100.0
A4	Initial pH	8.0	8.0	8.0	7.9	7.9	8.0
	96h OD $\pm$ SD (n=3) <sup>a</sup>	0.095 $\pm$ 0.002	0.092 $\pm$ 0.001	0.091 $\pm$ 0.001	0.089 $\pm$ 0.001	0.072 $\pm$ 0.002	0.014 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	63.1 $\pm$ 1.5	60.8 $\pm$ 0.8	60.1 $\pm$ 0.8	58.5 $\pm$ 0.8	45.6 $\pm$ 1.3	1.5 $\pm$ 0.8
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	65.0 $\pm$ 4.2	63.0 $\pm$ 4.5	58.0 $\pm$ 3.4	59.0 $\pm$ 4.7	43.0 $\pm$ 2.2	1.3 $\pm$ 0.5

Note :

@ : OD determined by Multiskan MCC/340 MKII

\* : cell count  $\times 10^4$  cells.mL<sup>-1</sup>

a : estimated from  $OD_{650\text{nm}}$  by calculation using regression equations

$$C. calcitrans : y = 1735x - 6.9$$

$$I. galbana : y = 3989.4x - 22.0$$

$$T. tetrahele : y = 647.4x - 3.8$$

$$T. sp. : y = 761x - 9.2$$

b : determined by direct cell count of a randomly selected test sample from triplicate, using haemocytometer

## Appendix 8(2)

Appendix 8 (2) : Raw data of cadmium range-finding tests with four selected marine phytoplankton, in the absence of EDTA

a) *C. calcitrans*

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	0.01	0.1	1.0	10.0	100.0
B1	Initial pH	8.1	7.9	7.9	7.9	8.1	8.0
	96h OD $\pm$ SD (n=3) @	0.173 $\pm$ 0.003	0.139 $\pm$ 0.008	0.055 $\pm$ 0.003	0.038 $\pm$ 0.002	0.012 $\pm$ 0.001	0.005 $\pm$ 0.001
	Estimated* Cell Count $\pm$ SD* (n=3)	293.2 $\pm$ 5.2	234.3 $\pm$ 13.1	88.5 $\pm$ 4.6	59.0 $\pm$ 3.5	13.9 $\pm$ 1.8	1.8 $\pm$ 1.7
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	294.5 $\pm$ 6.6	232.5 $\pm$ 2.4	86.8 $\pm$ 2.5	58.8 $\pm$ 2.8	14.3 $\pm$ 1.0	1.5 $\pm$ 0.6

b) *I. galbana*

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	0.01	0.1	1.0	10.0	100.0
B2	Initial pH	7.9	7.9	8.0	8.0	8.0	8.0
	96h OD $\pm$ SD (n=3) @	0.040 $\pm$ 0.003	0.031 $\pm$ 0.002	0.010 $\pm$ 0.001	0.007 $\pm$ 0.001	0.006 $\pm$ 0.001	0.006 $\pm$ 0.001
	Estimated* Cell Count $\pm$ SD* (n=3)	137.6 $\pm$ 10.6	101.7 $\pm$ 6.9	17.9 $\pm$ 4.0	7.2 $\pm$ 2.3	3.2 $\pm$ 2.3	3.2 $\pm$ 2.3
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	134.3 $\pm$ 2.6	102.5 $\pm$ 1.9	15.0 $\pm$ 2.6	6.5 $\pm$ 1.3	3.3 $\pm$ 1.0	2.8 $\pm$ 0.5

c) *T. tetrahele*

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	0.01	0.1	1.0	10.0	100.0
B3	Initial pH	8.0	8.0	8.1	8.0	8.0	8.0
	96h OD $\pm$ SD (n=3) @	0.060 $\pm$ 0.002	0.056 $\pm$ 0.001	0.055 $\pm$ 0.001	0.044 $\pm$ 0.001	0.023 $\pm$ 0.002	0.008 $\pm$ 0.001
	Estimated* Cell Count $\pm$ SD* (n=3)	35.0 $\pm$ 1.3	32.5 $\pm$ 0.7	31.8 $\pm$ 0.7	24.7 $\pm$ 0.7	11.1 $\pm$ 1.2	1.1 $\pm$ 0.5
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	33.5 $\pm$ 2.1	32.3 $\pm$ 1.3	33.5 $\pm$ 1.9	23.3 $\pm$ 2.2	11.0 $\pm$ 1.8	1.5 $\pm$ 0.7

d) *T. sp.*

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	0.01	0.1	1.0	10.0	100.0
B4	Initial pH	8.0	7.9	7.9	7.9	7.9	8.0
	96h OD $\pm$ SD (n=3) @	0.063 $\pm$ 0.003	0.060 $\pm$ 0.001	0.059 $\pm$ 0.002	0.053 $\pm$ 0.001	0.027 $\pm$ 0.001	0.015 $\pm$ 0.001
	Estimated* Cell Count $\pm$ SD* (n=3)	38.7 $\pm$ 2.0	36.5 $\pm$ 0.8	35.7 $\pm$ 1.3	31.1 $\pm$ 0.8	11.3 $\pm$ 0.8	2.0 $\pm$ 0.4
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	39.3 $\pm$ 2.5	35.0 $\pm$ 2.0	34.5 $\pm$ 2.1	31.0 $\pm$ 0.8	10.8 $\pm$ 1.3	2.2 $\pm$ 0.5

Note :

@ : OD determined by Multiskan MCC/340 MKII

\* : cell count  $\times 10^4$  cells.mL<sup>-1</sup>

a : estimated from OD<sub>max</sub> by calculation using regression equations

*C. calcitrans* :  $y=1735x-6.9$

*I. galbana* :  $y=3989.4x-22.0$

*T. tetrahele* :  $y=647.4x-3.8$

*T. sp.* :  $y=761x-9.2$

b : determined by direct cell count of a randomly selected test sample from triplicate, using haemocytometer

Appendix 8 (3) : Raw data of copper range-finding tests with four selected marine phytoplankton, in the presence of EDTA

a) *C. calcitrans*

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	0.01	0.1	1.0	10.0	100.0
C1	Initial pH	7.9	7.9	7.9	7.9	7.9	7.9
	96h OD $\pm$ SD (n=3) @	0.125 $\pm$ 0.002	0.120 $\pm$ 0.004	0.115 $\pm$ 0.003	0.111 $\pm$ 0.003	0.007 $\pm$ 0.001	0.005 $\pm$ 0.001
	Estimated* Cell Count $\pm$ SD* (n=3)	210.0 $\pm$ 3.5	201.3 $\pm$ 6.2	192.6 $\pm$ 4.6	185.7 $\pm$ 5.2	5.2 $\pm$ 1.8	1.8 $\pm$ 1.7
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	210.0 $\pm$ 3.7	201.3 $\pm$ 2.5	191.8 $\pm$ 1.7	184.8 $\pm$ 2.8	5.3 $\pm$ 1.0	1.7 $\pm$ 0.6

b) *I. galbana*

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	0.01	0.1	1.0	10.0	100.0
C2	Initial pH	7.9	7.9	7.9	7.9	7.9	8.0
	96h OD $\pm$ SD (n=3) @	0.055 $\pm$ 0.002	0.054 $\pm$ 0.001	0.052 $\pm$ 0.002	0.051 $\pm$ 0.001	0.008 $\pm$ 0.001	0.006 $\pm$ 0.001
	Estimated* Cell Count $\pm$ SD* (n=3)	197.4 $\pm$ 6.9	193.4 $\pm$ 4.0	185.4 $\pm$ 8.0	181.5 $\pm$ 4.0	8.6 $\pm$ 2.3	3.2 $\pm$ 2.3
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	199.0 $\pm$ 2.4	193.4 $\pm$ 2.2	185.0 $\pm$ 1.8	181.5 $\pm$ 5.0	9.0 $\pm$ 1.4	3.0 $\pm$ 0.8

c) *T. tetrahele*

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	0.01	0.1	1.0	10.0	100.0
C3	Initial pH	7.9	8.0	8.1	7.9	7.9	8.0
	96h OD $\pm$ SD (n=3) @	0.135 $\pm$ 0.001	0.127 $\pm$ 0.001	0.121 $\pm$ 0.004	0.113 $\pm$ 0.002	0.018 $\pm$ 0.001	0.007 $\pm$ 0.001
	Estimated* Cell Count $\pm$ SD* (n=3)	83.6 $\pm$ 0.6	78.4 $\pm$ 0.7	74.5 $\pm$ 2.4	69.4 $\pm$ 1.2	7.9 $\pm$ 0.7	0.9 $\pm$ 0.4
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	82.5 $\pm$ 2.6	77.8 $\pm$ 2.8	74.5 $\pm$ 1.9	67.0 $\pm$ 3.9	7.5 $\pm$ 1.3	0.8 $\pm$ 0.5

d) *T. sp.*

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	0.01	0.1	1.0	10.0	100.0
C4	Initial pH	7.9	7.9	8.1	8.0	8.1	8.0
	96h OD $\pm$ SD (n=3) @	0.129 $\pm$ 0.001	0.120 $\pm$ 0.003	0.118 $\pm$ 0.006	0.089 $\pm$ 0.001	0.023 $\pm$ 0.001	0.013 $\pm$ 0.001
	Estimated* Cell Count $\pm$ SD* (n=3)	89.0 $\pm$ 0.9	82.1 $\pm$ 2.7	80.6 $\pm$ 4.3	58.5 $\pm$ 0.8	8.3 $\pm$ 0.8	1.0 $\pm$ 0.5
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	88.0 $\pm$ 3.2	82.0 $\pm$ 0.8	82.3 $\pm$ 1.5	57.3 $\pm$ 2.2	8.5 $\pm$ 0.6	0.8 $\pm$ 0.5

Note :

@ : OD determined by Multiskan MCC/340 MKII

\* : cell count  $\times 10^4$  cells.mL $^{-1}$

a : estimated from OD<sub>620nm</sub> by calculation using regression equations

*C. calcitrans* :  $y=1735x-6.9$

*I. galbana* :  $y=3989x-22.0$

*T. tetrahele* :  $y=647.4x-3.8$

*T. sp.* :  $y=761x-9.2$

b : determined by direct cell count of a randomly selected test sample from triplicate, using haemocytometer

## Appendix 8(4)

Appendix 8 (4) : Raw data of copper range-finding tests with four selected marine phytoplankton, in the absence of EDTA

### a) *C. calcitrans*

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	0.01	0.1	1.0	10.0	100.0
D1	Initial pH	7.9	7.9	7.9	8.0	8.0	8.0
	96h OD $\pm$ SD (n=3) <sup>a</sup>	0.083 $\pm$ 0.006	0.058 $\pm$ 0.004	0.033 $\pm$ 0.001	0.009 $\pm$ 0.001	0.005 $\pm$ 0.001	0.005 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	137.1 $\pm$ 10.8	93.7 $\pm$ 7.0	50.4 $\pm$ 1.8	8.7 $\pm$ 1.8	1.8 $\pm$ 1.7	1.8 $\pm$ 1.7
	Actual <sup>b</sup> Cell Count $\pm$ SD*(n=4)	135.0 $\pm$ 2.6	94.0 $\pm$ 2.2	53.5 $\pm$ 3.1	8.0 $\pm$ 1.8	2.3 $\pm$ 0.5	1.7 $\pm$ 0.6

### b) *I. galbana*

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	0.01	0.1	1.0	10.0	100.0
D2	Initial pH	7.9	8.0	7.9	8.0	7.9	8.0
	96h OD $\pm$ SD (n=3) <sup>a</sup>	0.045 $\pm$ 0.001	0.033 $\pm$ 0.002	0.018 $\pm$ 0.001	0.008 $\pm$ 0.001	0.006 $\pm$ 0.001	0.006 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	157.5 $\pm$ 4.0	109.7 $\pm$ 6.9	49.8 $\pm$ 4.0	8.6 $\pm$ 2.3	3.2 $\pm$ 2.3	3.2 $\pm$ 2.3
	Actual <sup>b</sup> Cell Count $\pm$ SD*(n=4)	156.3 $\pm$ 1.7	107.8 $\pm$ 2.6	50.8 $\pm$ 1.0	8.0 $\pm$ 1.0	3.3 $\pm$ 0.5	2.8 $\pm$ 1.0

### c) *T. tetrahele*

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	0.01	0.1	1.0	10.0	100.0
D3	Initial pH	8.0	8.0	8.0	8.0	8.0	8.0
	96h OD $\pm$ SD (n=3) <sup>a</sup>	0.060 $\pm$ 0.001	0.049 $\pm$ 0.002	0.038 $\pm$ 0.001	0.012 $\pm$ 0.001	0.009 $\pm$ 0.001	0.008 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	35.0 $\pm$ 0.7	27.9 $\pm$ 1.2	20.8 $\pm$ 0.6	4.0 $\pm$ 0.7	2.0 $\pm$ 0.7	1.4 $\pm$ 0.7
	Actual <sup>b</sup> Cell Count $\pm$ SD*(n=4)	33.8 $\pm$ 2.1	27.8 $\pm$ 3.3	19.3 $\pm$ 1.7	4.5 $\pm$ 1.3	1.8 $\pm$ 0.5	1.3 $\pm$ 0.6

### d) *T. sp.*

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	0.01	0.1	1.0	10.0	100.0
D4	Initial pH	7.9	7.9	8.0	8.0	8.1	8.1
	96h OD $\pm$ SD (n=3) <sup>a</sup>	0.090 $\pm$ 0.005	0.083 $\pm$ 0.001	0.075 $\pm$ 0.002	0.024 $\pm$ 0.003	0.015 $\pm$ 0.001	0.014 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	59.3 $\pm$ 4.0	54.0 $\pm$ 0.8	47.9 $\pm$ 1.3	9.1 $\pm$ 2.0	2.2 $\pm$ 0.8	1.5 $\pm$ 0.8
	Actual <sup>b</sup> Cell Count $\pm$ SD*(n=4)	59.3 $\pm$ 2.2	52.8 $\pm$ 1.7	47.3 $\pm$ 1.0	8.5 $\pm$ 1.3	2.8 $\pm$ 1.0	1.7 $\pm$ 0.6

Note :

<sup>a</sup> : OD determined by Multiskan MCC/340 MKII

<sup>\*</sup> : cell count  $\times 10^6$  cells.mL<sup>-1</sup>

a : estimated from OD<sub>650nm</sub> by calculation using regression equations

*C. calcitrans* :  $y=1735x-6.9$

*I. galbana* :  $y=3989.4x-22.0$

*T. tetrahele* :  $y=647.4x-3.8$

*T. sp.* :  $y=761x-9.2$

b : determined by direct cell count of a randomly selected test sample from triplicate, using haemocytometer

Appendix 8 (5) : Raw data of manganese range-finding tests with four selected marine phytoplankton, in the presence of EDTA

a) *C. calcitrans*

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	0.01	0.1	1.0	10.0	100.0
E1	Initial pH	7.9	7.9	7.9	8.0	8.0	8.0
	96h OD $\pm$ SD (n=3) @	0.170 $\pm$ 0.001	0.183 $\pm$ 0.003	0.200 $\pm$ 0.006	0.172 $\pm$ 0.003	0.150 $\pm$ 0.010	0.054 $\pm$ 0.001
	Estimated* Cell Count $\pm$ SD* (n=3)	288.1 $\pm$ 1.8	310.6 $\pm$ 4.6	340.1 $\pm$ 9.7	291.5 $\pm$ 4.6	253.4 $\pm$ 17.4	86.8 $\pm$ 1.7
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	287.8 $\pm$ 6.4	305.5 $\pm$ 9.8	342.3 $\pm$ 4.2	291.5 $\pm$ 5.5	250.0 $\pm$ 7.3	85.0 $\pm$ 2.6

b) *I. galbana*

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	0.01	0.1	1.0	10.0	100.0
E2	Initial pH	7.9	7.9	8.0	8.0	8.0	8.1
	96h OD $\pm$ SD (n=3) @	0.081 $\pm$ 0.001	0.085 $\pm$ 0.001	0.090 $\pm$ 0.001	0.079 $\pm$ 0.002	0.064 $\pm$ 0.002	0.019 $\pm$ 0.001
	Estimated* Cell Count $\pm$ SD* (n=3)	301.1 $\pm$ 4.0	317.1 $\pm$ 4.0	337.0 $\pm$ 4.0	293.1 $\pm$ 6.0	233.3 $\pm$ 6.9	53.8 $\pm$ 4.0
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	300.3 $\pm$ 6.8	317.8 $\pm$ 4.2	336.5 $\pm$ 4.4	291.5 $\pm$ 4.8	238.5 $\pm$ 7.6	52.3 $\pm$ 5.1

c) *T. tetrahele*

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	0.01	0.1	1.0	10.0	100.0
E3	Initial pH	8.0	7.9	8.0	7.9	7.9	8.0
	96h OD $\pm$ SD (n=3) @	0.118 $\pm$ 0.002	0.124 $\pm$ 0.001	0.134 $\pm$ 0.001	0.116 $\pm$ 0.004	0.108 $\pm$ 0.004	0.049 $\pm$ 0.001
	Estimated* Cell Count $\pm$ SD* (n=3)	72.6 $\pm$ 1.2	76.5 $\pm$ 0.7	83.0 $\pm$ 0.7	71.3 $\pm$ 2.3	66.1 $\pm$ 2.8	27.9 $\pm$ 0.7
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	71.8 $\pm$ 3.0	75.0 $\pm$ 3.5	80.3 $\pm$ 3.3	70.8 $\pm$ 2.8	67.0 $\pm$ 2.6	27.3 $\pm$ 2.2

d) *T. sp.*

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	0.01	0.1	1.0	10.0	100.0
E4	Initial pH	7.9	8.0	8.0	7.9	8.0	8.0
	96h OD $\pm$ SD (n=3) @	0.127 $\pm$ 0.001	0.136 $\pm$ 0.005	0.150 $\pm$ 0.001	0.125 $\pm$ 0.002	0.123 $\pm$ 0.002	0.048 $\pm$ 0.001
	Estimated* Cell Count $\pm$ SD* (n=3)	87.4 $\pm$ 0.8	94.3 $\pm$ 3.5	105.0 $\pm$ 0.8	85.9 $\pm$ 1.3	84.4 $\pm$ 1.3	27.3 $\pm$ 0.8
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	85.8 $\pm$ 1.7	94.8 $\pm$ 2.5	105.0 $\pm$ 2.6	85.0 $\pm$ 3.0	85.5 $\pm$ 3.0	27.0 $\pm$ 2.6

Note :

@ : OD determined by Multiskan MCC/340 MKII

\* : cell count  $\times 10^4$  cells.mL<sup>-1</sup>

a : estimated from OD<sub>max</sub> by calculation using regression equations

*C. calcitrans* :  $y=1735x-6.9$

*I. galbana* :  $y=3989.4x-22.0$

*T. tetrahele* :  $y=647.4x-3.8$

*T. sp.* :  $y=761x-9.2$

b : determined by direct cell count of a randomly selected test sample from triplicate, using haemocytometer

Appendix 8 (6) : Raw data of manganese range-finding tests with four selected marine phytoplankton, in the absence of EDTA

a) *C. calcitrans*

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	0.01	0.1	1.0	10.0	100.0
F1	Initial pH	8.0	8.0	8.0	8.0	8.0	8.0
	96h OD $\pm$ SD (n=3) <sup>a</sup>	0.103 $\pm$ 0.002	0.108 $\pm$ 0.002	0.113 $\pm$ 0.003	0.099 $\pm$ 0.003	0.074 $\pm$ 0.001	0.025 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD <sup>a</sup> (n=3)	171.8 $\pm$ 3.0	180.5 $\pm$ 3.0	189.2 $\pm$ 4.6	164.9 $\pm$ 4.6	121.7 $\pm$ 1.7	36.5 $\pm$ 1.8
	Actual <sup>b</sup> Cell Count $\pm$ SD <sup>b</sup> (n=4)	172.5 $\pm$ 2.1	179.0 $\pm$ 2.6	190.3 $\pm$ 3.3	163.3 $\pm$ 2.8	121.0 $\pm$ 2.6	36.5 $\pm$ 1.9

b) *I. galbana*

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	0.01	0.1	1.0	10.0	100.0
F2	Initial pH	8.0	8.0	8.0	8.0	7.9	8.1
	96h OD $\pm$ SD (n=3) <sup>a</sup>	0.031 $\pm$ 0.002	0.032 $\pm$ 0.002	0.033 $\pm$ 0.001	0.029 $\pm$ 0.002	0.015 $\pm$ 0.001	0.008 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD <sup>a</sup> (n=3)	101.7 $\pm$ 6.9	105.7 $\pm$ 6.9	109.7 $\pm$ 4.0	93.7 $\pm$ 6.9	37.8 $\pm$ 4.0	8.6 $\pm$ 2.3
	Actual <sup>b</sup> Cell Count $\pm$ SD <sup>b</sup> (n=4)	103.8 $\pm$ 0.5	105.3 $\pm$ 2.2	109.0 $\pm$ 2.6	94.3 $\pm$ 1.7	36.3 $\pm$ 2.1	8.8 $\pm$ 1.9

c) *T. tetrahele*

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	0.01	0.1	1.0	10.0	100.0
F3	Initial pH	7.9	7.9	7.9	8.0	8.0	8.0
	96h OD $\pm$ SD (n=3) <sup>a</sup>	0.076 $\pm$ 0.002	0.079 $\pm$ 0.001	0.082 $\pm$ 0.003	0.074 $\pm$ 0.002	0.063 $\pm$ 0.001	0.019 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD <sup>a</sup> (n=3)	45.4 $\pm$ 1.1	47.3 $\pm$ 0.7	49.3 $\pm$ 1.7	44.1 $\pm$ 1.2	37.0 $\pm$ 0.7	8.5 $\pm$ 0.6
	Actual <sup>b</sup> Cell Count $\pm$ SD <sup>b</sup> (n=4)	44.7 $\pm$ 3.1	44.5 $\pm$ 2.8	49.0 $\pm$ 2.6	44.5 $\pm$ 1.9	36.0 $\pm$ 2.8	8.5 $\pm$ 1.3

d) *T. sp.*

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	0.01	0.1	1.0	10.0	100.0
F4	Initial pH	7.9	7.9	7.9	8.0	8.0	8.1
	96h OD $\pm$ SD (n=3) <sup>a</sup>	0.069 $\pm$ 0.004	0.072 $\pm$ 0.001	0.076 $\pm$ 0.002	0.067 $\pm$ 0.004	0.063 $\pm$ 0.001	0.019 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD <sup>a</sup> (n=3)	43.3 $\pm$ 2.7	45.6 $\pm$ 0.8	48.6 $\pm$ 1.6	41.8 $\pm$ 2.8	38.7 $\pm$ 0.8	5.3 $\pm$ 0.8
	Actual <sup>b</sup> Cell Count $\pm$ SD <sup>b</sup> (n=4)	44.7 $\pm$ 1.7	44.5 $\pm$ 1.9	49.0 $\pm$ 2.6	41.5 $\pm$ 1.7	38.8 $\pm$ 2.5	5.3 $\pm$ 1.0

Note :

@ : OD determined by Multiskan MCC/340 MKII

\* : cell count X 10<sup>6</sup> cells.mL<sup>-1</sup>

a : estimated from OD<sub>max</sub> by calculation using regression equations

*C. calcitrans* :  $y=1735x-6.9$

*I. galbana* :  $y=3989.4x-22.0$

*T. tetrahele* :  $y=647.4x-3.8$

*T. sp.* :  $y=761x-9.2$

b : determined by direct cell count of a randomly selected test sample from triplicate, using haemocytometer

## Appendix 8(7)

Appendix 8 (7) : Raw data of arsenic range-finding tests with four selected marine phytoplankton, in the presence of EDTA

### a) *C. calcitrans*

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	0.01	0.1	1.0	10.0	100.0
G1	Initial pH	7.9	7.9	8.0	8.0	8.0	8.0
	96h OD $\pm$ SD (n=3) @	0.100 $\pm$ 0.002	0.105 $\pm$ 0.001	0.112 $\pm$ 0.001	0.163 $\pm$ 0.003	0.176 $\pm$ 0.005	0.129 $\pm$ 0.002
	Estimated* Cell Count $\pm$ SD* (n=3)	166.6 $\pm$ 3.5	175.3 $\pm$ 1.8	187.4 $\pm$ 1.8	275.9 $\pm$ 10.6	298.5 $\pm$ 9.1	216.9 $\pm$ 3.0
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	166.3 $\pm$ 3.5	173.3 $\pm$ 2.2	186.0 $\pm$ 3.3	276.0 $\pm$ 11.8	298.0 $\pm$ 3.7	217.8 $\pm$ 2.5

### b) *I. galbana*

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	0.01	0.1	1.0	10.0	100.0
G2	Initial pH	7.9	8.0	8.0	8.0	8.0	8.0
	96h OD $\pm$ SD (n=3) @	0.044 $\pm$ 0.001	0.045 $\pm$ 0.001	0.047 $\pm$ 0.001	0.066 $\pm$ 0.003	0.071 $\pm$ 0.001	0.039 $\pm$ 0.001
	Estimated* Cell Count $\pm$ SD* (n=3)	153.5 $\pm$ 4.0	157.5 $\pm$ 4.0	165.5 $\pm$ 4.0	241.3 $\pm$ 10.6	261.2 $\pm$ 4.0	133.6 $\pm$ 4.0
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	154.0 $\pm$ 2.0	158.7 $\pm$ 4.2	164.5 $\pm$ 3.0	241.5 $\pm$ 4.4	260.0 $\pm$ 6.3	134.3 $\pm$ 2.6

### c) *T. tetrahele*

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	0.01	0.1	1.0	10.0	100.0
G3	Initial pH	7.9	8.0	8.0	8.0	8.0	8.1
	96h OD $\pm$ SD (n=3) @	0.145 $\pm$ 0.001	0.150 $\pm$ 0.002	0.155 $\pm$ 0.002	0.138 $\pm$ 0.002	0.113 $\pm$ 0.002	0.059 $\pm$ 0.003
	Estimated* Cell Count $\pm$ SD* (n=3)	90.1 $\pm$ 0.7	93.3 $\pm$ 1.2	96.5 $\pm$ 1.1	85.5 $\pm$ 1.2	69.4 $\pm$ 1.2	34.4 $\pm$ 2.3
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	93.7 $\pm$ 2.5	93.3 $\pm$ 2.2	94.0 $\pm$ 2.8	83.3 $\pm$ 2.2	73.5 $\pm$ 1.9	35.0 $\pm$ 2.6

### d) *T. sp.*

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	0.01	0.1	1.0	10.0	100.0
G4	Initial pH	7.9	7.9	8.0	7.9	8.0	8.1
	96h OD $\pm$ SD (n=3) @	0.121 $\pm$ 0.002	0.127 $\pm$ 0.002	0.130 $\pm$ 0.006	0.119 $\pm$ 0.002	0.103 $\pm$ 0.003	0.049 $\pm$ 0.004
	Estimated* Cell Count $\pm$ SD* (n=3)	82.9 $\pm$ 1.3	87.4 $\pm$ 1.6	89.7 $\pm$ 4.7	81.4 $\pm$ 1.3	69.2 $\pm$ 2.6	28.1 $\pm$ 2.8
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	82.8 $\pm$ 1.5	84.5 $\pm$ 2.5	89.0 $\pm$ 4.8	84.3 $\pm$ 1.7	70.7 $\pm$ 3.2	31.0 $\pm$ 3.7

Note :

@ : OD determined by Multiskan MCC/340 MKII

\* : cell count  $\times 10^6$  cells.mL<sup>-1</sup>

a : estimated from OD<sub>96h</sub> by calculation using regression equations

*C. calcitrans* :  $y = 1735x - 9.9$

*I. galbana* :  $y = 3989.4x - 22.0$

*T. tetrahele* :  $y = 647.4x - 3.8$

*T. sp.* :  $y = 761x - 9.2$

b : determined by direct cell count of a randomly selected test sample from triplicate, using haemocytometer

Appendix 8 (8) : Raw data of arsenic range-finding tests with four selected marine phytoplankton, in the absence of EDTA

a) *C. calcitrans*

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	0.01	0.1	1.0	10.0	100.0
H1	Initial pH	7.8	7.9	7.9	7.9	8.0	8.0
	96h OD $\pm$ SD (n=3) *	0.075 $\pm$ 0.002	0.079 $\pm$ 0.001	0.082 $\pm$ 0.003	0.107 $\pm$ 0.001	0.111 $\pm$ 0.004	0.068 $\pm$ 0.004
	Estimated* Cell Count $\pm$ SD* (n=3)	123.2 $\pm$ 3.0	130.2 $\pm$ 1.8	135.4 $\pm$ 4.6	178.7 $\pm$ 1.8	185.7 $\pm$ 6.3	111.1 $\pm$ 7.0
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	122.8 $\pm$ 2.2	133.3 $\pm$ 4.2	135.3 $\pm$ 2.5	172.8 $\pm$ 3.6	185.8 $\pm$ 4.0	111.0 $\pm$ 2.6

b) *I. galbana*

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	0.01	0.1	1.0	10.0	100.0
H2	Initial pH	8.0	8.0	8.0	8.0	7.9	7.9
	96h OD $\pm$ SD (n=3) *	0.042 $\pm$ 0.001	0.044 $\pm$ 0.001	0.045 $\pm$ 0.001	0.057 $\pm$ 0.003	0.063 $\pm$ 0.003	0.037 $\pm$ 0.003
	Estimated* Cell Count $\pm$ SD* (n=3)	145.6 $\pm$ 4.0	153.5 $\pm$ 4.0	157.5 $\pm$ 4.0	205.4 $\pm$ 10.6	229.3 $\pm$ 12.0	125.6 $\pm$ 10.6
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	143.5 $\pm$ 1.9	148.3 $\pm$ 5.7	156.5 $\pm$ 4.4	204.8 $\pm$ 3.0	228.0 $\pm$ 4.1	125.0 $\pm$ 2.6

c) *T. tetrahele*

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	0.01	0.1	1.0	10.0	100.0
H3	Initial pH	7.9	7.9	7.9	7.9	7.9	7.9
	96h OD $\pm$ SD (n=3) *	0.112 $\pm$ 0.007	0.116 $\pm$ 0.002	0.120 $\pm$ 0.009	0.107 $\pm$ 0.006	0.070 $\pm$ 0.001	0.042 $\pm$ 0.003
	Estimated* Cell Count $\pm$ SD* (n=3)	68.7 $\pm$ 4.5	71.3 $\pm$ 1.1	73.9 $\pm$ 5.7	65.5 $\pm$ 4.0	41.9 $\pm$ 0.7	23.4 $\pm$ 1.7
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	67.8 $\pm$ 4.6	73.0 $\pm$ 2.6	73.0 $\pm$ 1.8	65.0 $\pm$ 2.6	43.8 $\pm$ 1.7	23.0 $\pm$ 1.8

d) *T. sp.*

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	0.01	0.1	1.0	10.0	100.0
H4	Initial pH	7.9	7.9	8.0	8.0	8.1	8.1
	96h OD $\pm$ SD (n=3) *	0.087 $\pm$ 0.002	0.089 $\pm$ 0.004	0.092 $\pm$ 0.003	0.085 $\pm$ 0.001	0.066 $\pm$ 0.004	0.021 $\pm$ 0.001
	Estimated* Cell Count $\pm$ SD* (n=3)	57.0 $\pm$ 1.3	58.5 $\pm$ 3.1	60.8 $\pm$ 2.7	55.5 $\pm$ 0.8	41.0 $\pm$ 3.1	6.8 $\pm$ 0.8
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	55.5 $\pm$ 3.7	57.0 $\pm$ 1.8	60.8 $\pm$ 2.2	55.3 $\pm$ 2.2	40.2 $\pm$ 2.5	6.3 $\pm$ 1.7

Note :

@ : OD determined by Multiskan MCC/340 MKII

\* : cell count  $\times 10^4$  cells.mL<sup>-1</sup>

a : estimated from OD<sub>max</sub> by calculation using regression equations

*C. calcitrans* :  $y=1735x-6.9$

*I. galbana* :  $y=3989.4x-22.0$

*T. tetrahele* :  $y=647.4x-3.8$

*T. sp.* :  $y=761x-9.2$

b : determined by direct cell count of a randomly selected test sample from triplicate, using haemocytometer

Appendix 9 (1a) : Raw data of cadmium toxicity tests with *Chaetoceros calcitrans*, conducted in multiwell plates, in the presence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	6.25	12.5	25.0	50.0	100.0
A1.1	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	NA	5.8	11.8	23.7	47.7	97.4
	Initial pH	8.2	8.2	8.2	8.2	8.1	8.1
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.163 $\pm$ 0.009	0.128 $\pm$ 0.004	0.087 $\pm$ 0.008	0.007 $\pm$ 0.001	0.007 $\pm$ 0.001	0.007 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD <sup>b</sup> (n=3)	276.5 $\pm$ 16.1	215.2 $\pm$ 7.0	144.0 $\pm$ 13.6	5.2 $\pm$ 1.8	5.2 $\pm$ 1.8	4.6 $\pm$ 1.0
A1.2	Actual <sup>b</sup> Cell Count $\pm$ SD <sup>b</sup> (n=4)	297.8 $\pm$ 6.1	199.0 $\pm$ 7.9	151.0 $\pm$ 5.5	6.8 $\pm$ 1.5	3.5 $\pm$ 0.6	3.0 $\pm$ 0.8
	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0004	5.0	12.5	22.6	38.4	104.4
	Initial pH	8.1	8.2	8.2	8.2	8.2	8.1
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.124 $\pm$ 0.007	0.102 $\pm$ 0.008	0.096 $\pm$ 0.003	0.012 $\pm$ 0.002	0.012 $\pm$ 0.002	0.011 $\pm$ 0.001
A1.2	Estimated <sup>a</sup> Cell Count $\pm$ SD <sup>b</sup> (n=3)	208.3 $\pm$ 11.4	170.1 $\pm$ 13.6	159.1 $\pm$ 5.3	14.5 $\pm$ 2.7	13.4 $\pm$ 2.6	11.6 $\pm$ 1.0
	Actual <sup>b</sup> Cell Count $\pm$ SD <sup>b</sup> (n=4)	206.8 $\pm$ 4.6	187.8 $\pm$ 5.0	160.5 $\pm$ 5.8	13.8 $\pm$ 2.2	10.5 $\pm$ 1.9	11.5 $\pm$ 2.9

Appendix 9 (1b) : Raw data of cadmium toxicity tests with *Chaetoceros calcitrans*, conducted in flasks, in the presence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	6.25	12.5	25.0	50.0	100.0
A1.3	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0004	5.0	12.5	22.6	38.4	104.4
	Initial pH	8	7.9	7.9	7.9	7.9	7.9
	Final pH (n=3)	9.39 $\pm$ 0.03	9.40 $\pm$ 0.02	9.27 $\pm$ 0.04	8.02 $\pm$ 0.01	7.88 $\pm$ 0.01	7.86 $\pm$ 0.01
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.115 $\pm$ 0.007	0.105 $\pm$ 0.003	0.093 $\pm$ 0.001	0.018 $\pm$ 0.001	0.007 $\pm$ 0.001	0.005 $\pm$ 0.001
A1.4	Estimated <sup>a</sup> Cell Count $\pm$ SD <sup>b</sup> (n=3)	193.2 $\pm$ 12.2	173.3 $\pm$ 4.6	154.5 $\pm$ 1.8	23.8 $\pm$ 2.0	4.6 $\pm$ 1.0	2.4 $\pm$ 1.0
	Actual <sup>b</sup> Cell Count $\pm$ SD <sup>b</sup> (n=4)	203.5 $\pm$ 11.5	176.0 $\pm$ 10.5	156.8 $\pm$ 4.8	25.3 $\pm$ 3.1	4.8 $\pm$ 1.3	1.3 $\pm$ 0.5
A1.4	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	NA	NA	NA	NA	NA	NA
	Initial pH	8.0	7.9	7.9	7.9	7.9	7.9
	Final pH (n=3)	9.89 $\pm$ 0.03	9.40 $\pm$ 0.45	8.50 $\pm$ 0.21	8.32 $\pm$ 0.57	7.87 $\pm$ 0.02	7.84 $\pm$ 0.01
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.084 $\pm$ 0.005	0.062 $\pm$ 0.003	0.028 $\pm$ 0.006	0.006 $\pm$ 0.001	0.006 $\pm$ 0.001	0.005 $\pm$ 0.001
A1.4	Estimated <sup>a</sup> Cell Count $\pm$ SD <sup>b</sup> (n=3)	138.8 $\pm$ 9.7	101.3 $\pm$ 4.4	47.4 $\pm$ 11.0	4.1 $\pm$ 1.0	2.9 $\pm$ 1.0	1.0 $\pm$ 0.8
	Actual <sup>b</sup> Cell Count $\pm$ SD <sup>b</sup> (n=4)	139.8 $\pm$ 9.3	105.5 $\pm$ 6.8	50.0 $\pm$ 8.5	4.5 $\pm$ 1.3	2.5 $\pm$ 1.0	1.0 $\pm$ 0.8

Note :

# : Actual initial heavy metal concentration determined by ICP-AES (NA=not available)

@ : OD determined by Multiskan MCC/340 MKII

\* : cell count X 10<sup>4</sup> cells.mL<sup>-1</sup>

a : estimated from OD<sub>620nm</sub> by calculation using regression equation :  $y=1735x-6.9$

b : determined by direct cell count of a randomly selected test sample from triplicate, using haemocytometer

Appendix 9 (2a) : Raw data of cadmium toxicity tests with *Isochrysis galbana*, conducted in multiwell plates, in the presence of EDTA.

Exp. No.	Nominal Initial Test Concentration (mgL <sup>-1</sup> )	0	6.25	12.5	25.0	50.0	100.0
A2.1	Actual Initial Test Conc. (mgL <sup>-1</sup> ) <sup>#</sup>	NA	5.8	11.8	23.7	47.8	97.4
	Initial pH	8.2	8.2	8.2	8.2	8.1	8.1
	96h OD ± SD (n=3) <sup>@</sup>	0.064±0.002	0.022±0.002	0.010±0.001	0.006±0.001	0.006±0.001	0.006±0.001
	Estimated <sup>a</sup> Cell Count ± SD* (n=3)	232.0±6.1	64.4±8.3	16.6±2.3	4.6±2.3	3.2±2.3	3.2±2.3
	Actual <sup>b</sup> Cell Count ± SD* (n=4)	219.8±4.1	60.3±4.5	15.8±2.4	2.8±1.0	2.5±0.6	1.5±0.6

Appendix 9 (2b) : Raw data of cadmium toxicity tests with *Isochrysis galbana*, conducted in flasks, in the presence of EDTA

Exp. No.	Nominal Initial Test Concentration (mgL <sup>-1</sup> )	0	6.25	12.5	25.0	50.0	100.0
A2.2	Actual Initial Test Conc. (mgL <sup>-1</sup> ) <sup>#</sup>	0.0004	5.0	12.5	22.6	38.4	104.4
	Initial pH	8.1	8.2	8.2	8.2	8.2	8.1
	Final pH (n=3)	8.67±0.01	8.42±0.01	7.88±0.01	7.88±0.02	7.87±0.02	7.84±0.02
	96h OD ± SD (n=3) <sup>@</sup>	0.055±0.002	0.032±0.001	0.009±0.001	0.008±0.001	0.008±0.001	0.007±0.001
	Estimated <sup>a</sup> Cell Count ± SD* (n=3)	197.4±8.0	104.4±4.6	12.6±2.3	11.2±2.3	8.6±2.3	3.2±2.3
	Actual <sup>b</sup> Cell Count ± SD* (n=4)	199.8±7.3	100.3±5.6	1.5±0.6	1.3±0.5	1.0±0.8	0.8±0.5

Note :

# : Actual initial heavy metal concentration determined by ICP-AES

@ : OD determined by Multikan MCC/340 MKII

\* : cell count X 10<sup>4</sup> cells.mL<sup>-1</sup>

a : estimated from OD<sub>620nm</sub> by calculation using regression equation : y=3989.4x-22.0

b : determined by direct cell count of a randomly selected test sample from triplicate, using haemocytometer

Appendix 9 (3a) : Raw data of cadmium toxicity tests with *Tetraselmis tetrahele*, conducted in multiwell plates, in the presence of EDTA

Exp. No.	Nominal Initial Test Concentration (mgL <sup>-1</sup> )	0	6.25	12.5	25.0	50.0	100.0
A3.2	Actual Initial Test Conc. (mgL <sup>-1</sup> ) <sup>#</sup>	NA	5.8	11.8	23.7	47.7	97.4
	Initial pH	8.2	8.2	8.2	8.2	8.1	8.1
	96h OD ± SD (n=3) <sup>a</sup>	0.056±0.001	0.045±0.003	0.052±0.008	0.018±0.002	0.016±0.003	0.012±0.002
	Estimated* Cell Count ± SD* (n=3)	32.5±0.7	25.1±5.6	29.9±5.1	8.1±1.0	6.6±1.7	3.2±0.6
	Actual <sup>b</sup> Cell Count ± SD* (n=4)	34.8±2.6	29.3±5.1	33.8±3.3	7.8±1.7	6.5±1.3	3.3±1.3

Appendix 9 (3b) : Raw data of cadmium toxicity tests with *Tetraselmis tetrahele*, conducted in flasks, in the presence of EDTA

Exp No.	Nominal Initial Test Concentration (mgL <sup>-1</sup> )	0	6.25	12.5	25.0	50.0	100.0
A3.3	Actual Initial Test Conc. (mgL <sup>-1</sup> ) <sup>#</sup>	NA	NA	NA	NA	NA	NA
	Initial pH	8.0	7.9	7.9	7.9	7.9	7.9
	Final pH (n=3)	9.69±0.04	9.69±0.01	9.71±0.03	8.59±0.13	8.01±0.03	7.90±0.01
	96h OD ± SD (n=3) <sup>a</sup>	0.085±0.007	0.093±0.003	0.064±0.001	0.022±0.003	0.008±0.001	0.007±0.001
	Estimated* Cell Count ± SD* (n=3)	51.0±4.7	56.2±1.9	37.9±0.8	10.6±2.1	1.2±0.4	0.9±0.4
	Actual <sup>b</sup> Cell Count ± SD* (n=4)	48.0±5.4	52.8±3.3	34.0±1.8	10.5±2.6	1.7±0.6	1.3±0.5

Note :

# : Actual initial heavy metal concentration determined by ICP-AES (NA=not available)

@ : OD determined by Multiskan MCC/340 MKII

\* : cell count X 10<sup>6</sup> cells.mL<sup>-1</sup>

a : estimated from OD<sub>650nm</sub> by calculation using regression equation : y=647.4x-3.8

b : determined by direct cell count of a randomly selected test sample from triplicate, using haemocytometer

## Appendix 9(4)

**Appendix 9 (4a) :** Raw data of cadmium toxicity tests with *Tetraselmis* sp., conducted in multiwell plates, in the presence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	6.25	12.5	25.0	50.0	100.0
A4.1	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0002	6.2	12.5	25.3	50.8	103.2
	Initial pH	8.0	7.7	7.8	7.8	7.7	7.6
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.136 $\pm$ 0.013	0.088 $\pm$ 0.012	0.072 $\pm$ 0.006	0.020 $\pm$ 0.003	0.014 $\pm$ 0.001	0.014 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	94.3 $\pm$ 9.9	57.7 $\pm$ 8.8	45.3 $\pm$ 4.4	6.0 $\pm$ 2.0	1.7 $\pm$ 0.4	1.2 $\pm$ 0.5
A4.2	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	87.3 $\pm$ 2.2	44.0 $\pm$ 1.8	38.8 $\pm$ 2.8	5.3 $\pm$ 1.7	2.8 $\pm$ 1.0	1.3 $\pm$ 0.5
	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	NA	5.8	11.8	23.7	47.7	97.4
	Initial pH	8.2	8.2	8.2	8.2	8.1	8.1
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.058 $\pm$ 0.006	0.053 $\pm$ 0.002	0.049 $\pm$ 0.005	0.015 $\pm$ 0.001	0.014 $\pm$ 0.001	0.014 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	35.2 $\pm$ 4.3	30.9 $\pm$ 1.2	27.8 $\pm$ 3.6	2.0 $\pm$ 0.4	1.7 $\pm$ 0.4	1.2 $\pm$ 0.5
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	33.5 $\pm$ 1.9	30.0 $\pm$ 3.6	24.8 $\pm$ 1.7	3.0 $\pm$ 0.8	2.5 $\pm$ 1.3	1.0 $\pm$ 0.8

**Appendix 9 (4b) :** Raw data of cadmium toxicity tests with *Tetraselmis* sp., conducted in flasks, in the presence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	6.25	12.5	25.0	50.0	100.0
A4.3	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	NA	NA	NA	NA	NA	NA
	Initial pH	8.0	8.0	8.0	7.9	7.9	7.9
	Final pH (n=3)	9.66 $\pm$ 0.04	9.68 $\pm$ 0.02	9.68 $\pm$ 0.04	8.35 $\pm$ 0.05	7.96 $\pm$ 0.02	7.90 $\pm$ 0.02
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.098 $\pm$ 0.008	0.098 $\pm$ 0.007	0.089 $\pm$ 0.006	0.017 $\pm$ 0.002	0.014 $\pm$ 0.001	0.014 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	65.6 $\pm$ 6.2	65.6 $\pm$ 5.1	58.5 $\pm$ 4.6	3.5 $\pm$ 1.6	1.7 $\pm$ 0.4	1.2 $\pm$ 0.5
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	59.8 $\pm$ 5.1	63.8 $\pm$ 2.9	56.8 $\pm$ 2.8	2.8 $\pm$ 1.0	2.0 $\pm$ 0.8	1.5 $\pm$ 0.6

Note :

# : Actual initial heavy metal concentration determined by ICP-AES (NA= not available)

@ : OD determined by Multiskan MCC/340 MKII

\* : cell count  $\times 10^4$  cells  $\text{mL}^{-1}$

a : estimated from OD<sub>420nm</sub> by calculation using regression equation : y=761x-9.2

b : determined by direct cell count of a randomly selected test sample from triplicate, using haemocytometer

Appendix 10 (1) : Raw data of cadmium toxicity tests with *Chaetoceros calcitrans*, conducted in multiwell plates, in the absence of EDTA.

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	0.10	0.18	0.32	0.56	1.00
B1.1	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0003	0.09	0.16	0.27	0.52	0.99
	Initial pH	8.0	8.0	8.0	8.0	8.0	8.0
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.161 $\pm$ 0.010	0.028 $\pm$ 0.006	0.022 $\pm$ 0.001	0.018 $\pm$ 0.002	0.011 $\pm$ 0.002	0.008 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	273.0 $\pm$ 17.5	41.1 $\pm$ 9.6	30.5 $\pm$ 0.7	23.8 $\pm$ 2.6	12.7 $\pm$ 3.6	7.6 $\pm$ 1.0
B1.3	Actual <sup>b</sup> Cell Count $\pm$ SD*(n=4)	276.8 $\pm$ 7.1	38.3 $\pm$ 1.3	30.5 $\pm$ 1.9	19.5 $\pm$ 3.1	15.5 $\pm$ 1.9	8.0 $\pm$ 1.4
	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0002	0.08	0.16	0.30	0.63	0.90
	Initial pH	8.0	8.0	8.0	8.0	8.0	8.0
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.185 $\pm$ 0.009	0.085 $\pm$ 0.014	0.087 $\pm$ 0.007	0.092 $\pm$ 0.009	0.071 $\pm$ 0.004	0.069 $\pm$ 0.003
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	313.5 $\pm$ 16.1	140.6 $\pm$ 24.3	144.7 $\pm$ 11.8	152.7 $\pm$ 15.4	116.3 $\pm$ 7.0	114.0 $\pm$ 4.0
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	306.5 $\pm$ 4.5	146.5 $\pm$ 3.4	156.5 $\pm$ 4.1	160.3 $\pm$ 6.2	116.8 $\pm$ 4.0	114.0 $\pm$ 4.3

Note :

# : Actual initial heavy metal concentration determined by ICP-AES

@ : OD determined by Multiskan MCC/340 MKII

\* : cell count X  $10^4$  cells.mL<sup>-3</sup>

a : estimated from OD<sub>96h</sub> by calculation using regression equation :  $y = 1735x - 6.9$

b : determined by direct cell count of a randomly selected test sample from triplicate, using haemocytometer

## Appendix 10(2)

**Appendix 10 (2a) :** Raw data of cadmium toxicity tests with *Isochrysis galbana*, conducted in multiwell plates, in the absence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	0.10	0.18	0.32	0.56	1.00
B2.3	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0002	0.08	0.16	0.30	0.63	0.90
	Initial pH	8.0	8.0	8.0	8.0	8.0	8.0
	96h OD $\pm$ SD (n=3) <sup>a</sup>	0.048 $\pm$ 0.004	0.015 $\pm$ 0.003	0.012 $\pm$ 0.003	0.007 $\pm$ 0.001	0.006 $\pm$ 0.001	0.006 $\pm$ 0.001
	Estimated <sup>b</sup> Cell Count $\pm$ SD* (n=3)	170.8 $\pm$ 16.1	36.5 $\pm$ 11.5	24.6 $\pm$ 4.6	7.2 $\pm$ 2.3	3.2 $\pm$ 2.3	3.2 $\pm$ 2.3
B2.4	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	167.0 $\pm$ 2.8	30.5 $\pm$ 3.7	22.3 $\pm$ 2.1	8.0 $\pm$ 1.6	1.0 $\pm$ 0.8	1.0 $\pm$ 0.8
	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	NA	0.12	0.20	0.29	0.51	0.80
	Initial pH	8.0	8.1	8.1	8.1	8.1	8.0
	96h OD $\pm$ SD (n=3) <sup>a</sup>	0.027 $\pm$ 0.001	0.008 $\pm$ 0.001	0.008 $\pm$ 0.001	0.008 $\pm$ 0.001	0.007 $\pm$ 0.001	0.007 $\pm$ 0.001
	Estimated <sup>b</sup> Cell Count $\pm$ SD* (n=3)	84.4 $\pm$ 2.3	11.2 $\pm$ 2.3	8.6 $\pm$ 2.3	8.6 $\pm$ 2.3	7.2 $\pm$ 2.3	4.6 $\pm$ 2.3
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	82.5 $\pm$ 3.7	10.5 $\pm$ 3.4	8.5 $\pm$ 1.3	7.3 $\pm$ 2.1	9.3 $\pm$ 2.2	6.5 $\pm$ 1.3

**Appendix 10 (2b) :** Raw data of cadmium toxicity tests with *Isochrysis galbana*, conducted in flasks, in the absence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	0.10	0.18	0.32	0.56	1.00
B2.5	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	NA	0.12	0.20	0.29	0.51	0.80
	Initial pH	8.0	8.1	8.1	8.1	8.1	8.0
	Final pH (n=3)	8.79 $\pm$ 0.04	8.06 $\pm$ 0.05	8.06 $\pm$ 0.05	8.06 $\pm$ 0.05	8.10 $\pm$ 0.05	7.85 $\pm$ 0.04
	96h OD $\pm$ SD (n=3) <sup>a</sup>	0.044 $\pm$ 0.002	0.008 $\pm$ 0.001	0.008 $\pm$ 0.001	0.008 $\pm$ 0.001	0.007 $\pm$ 0.001	0.006 $\pm$ 0.001
	Estimated <sup>b</sup> Cell Count $\pm$ SD* (n=3)	154.8 $\pm$ 6.1	8.6 $\pm$ 2.3	8.6 $\pm$ 2.3	8.6 $\pm$ 2.3	4.6 $\pm$ 2.3	3.2 $\pm$ 2.3
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	154.0 $\pm$ 2.6	9.8 $\pm$ 2.8	8.5 $\pm$ 2.8	9.8 $\pm$ 2.8	6.3 $\pm$ 1.7	1.0 $\pm$ 0.8

Note :

# : Actual initial heavy metal concentration determined by ICP-AES

@ : OD determined by Multiskan MCC/340 MKII

\* : cell count  $\times 10^4$  cells. $\text{mL}^{-1}$

a : estimated from OD<sub>420nm</sub> by calculation using regression equation :  $y = 3989.4x - 22.0$

b : determined by direct cell count of a randomly selected test sample from triplicate, using haemocytometer

Appendix 10 (3a) : Raw data of cadmium toxicity tests with *Tetraselmis tetrahele*, conducted in multiwell plates, in the absence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	6.25	12.5	25.0	50.0	100.0
B3.1	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0003	6.1	12.3	24.8	49.8	97.0
	Initial pH	8.0	7.9	7.9	7.9	7.9	7.9
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.060 $\pm$ 0.005	0.031 $\pm$ 0.004	0.018 $\pm$ 0.004	0.012 $\pm$ 0.001	0.009 $\pm$ 0.001	0.007 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	34.8 $\pm$ 3.3	16.5 $\pm$ 2.4	7.6 $\pm$ 2.4	4.0 $\pm$ 0.7	2.0 $\pm$ 0.7	0.9 $\pm$ 0.4
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	29.8 $\pm$ 3.5	14.5 $\pm$ 2.1	5.3 $\pm$ 1.5	5.3 $\pm$ 1.0	1.5 $\pm$ 0.6	1.0 $\pm$ 0.8
B3.3	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0004	5.8	11.9	23.2	47.2	94.5
	Initial pH	8.0	8.0	8.0	8.0	7.9	7.9
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.071 $\pm$ 0.005	0.028 $\pm$ 0.001	0.024 $\pm$ 0.004	0.017 $\pm$ 0.001	0.008 $\pm$ 0.001	0.008 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	42.1 $\pm$ 3.3	14.1 $\pm$ 0.3	11.7 $\pm$ 2.3	7.0 $\pm$ 0.8	1.6 $\pm$ 0.3	1.2 $\pm$ 0.4
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	46.4 $\pm$ 3.2	15.5 $\pm$ 2.1	14.8 $\pm$ 2.1	5.8 $\pm$ 1.0	1.5 $\pm$ 0.6	0.8 $\pm$ 0.5

Appendix 10 (3b) : Raw data of cadmium toxicity tests with *Tetraselmis tetrahele*, conducted in flasks, in the absence of EDTA

Exp No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	6.25	12.5	25.0	50.0	100.0
B3.4	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	NA	6.1	11.0	25.8	51.7	102.4
	Initial pH	8.1	8.1	8.0	8.0	8.1	8.0
	Final pH (n=3)	9.18 $\pm$ 0.48	8.52 $\pm$ 0.12	8.25 $\pm$ 0.07	8.01 $\pm$ 0.01	7.89 $\pm$ 0.01	7.83 $\pm$ 0.02
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.043 $\pm$ 0.005	0.026 $\pm$ 0.002	0.018 $\pm$ 0.002	0.014 $\pm$ 0.001	0.010 $\pm$ 0.001	0.010 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	24.1 $\pm$ 3.3	12.8 $\pm$ 1.5	7.8 $\pm$ 1.1	5.1 $\pm$ 0.4	2.9 $\pm$ 0.3	2.5 $\pm$ 0.4
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	26.0 $\pm$ 1.8	9.8 $\pm$ 1.7	6.3 $\pm$ 1.0	5.3 $\pm$ 1.0	3.3 $\pm$ 1.0	1.0 $\pm$ 0.8

Note :

# : Actual initial heavy metal concentration determined by ICP-AES

@ : OD determined by Multiskan MCC/340 MKII

\* : cell count  $\times 10^6$  cells.mL<sup>-1</sup>

a : estimated from OD<sub>620nm</sub> by calculation using regression equation :  $y=647.4x-3.8$

b : determined by direct cell count of a randomly selected test sample from triplicate, using haemocytometer

## Appendix 10(4)

**Appendix 10 (4a) :** Raw data of cadmium toxicity tests with *Tetraselmis* sp., conducted in multiwell plates, in the absence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	6.25	12.5	25.0	50.0	100.0
B4.1	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0003	6.1	12.3	24.8	49.8	97.0
	Initial pH	8.0	7.9	7.9	7.9	7.9	7.9
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.055 $\pm$ 0.009	0.026 $\pm$ 0.006	0.019 $\pm$ 0.003	0.014 $\pm$ 0.001	0.014 $\pm$ 0.001	0.015 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	32.7 $\pm$ 6.6	10.6 $\pm$ 4.6	5.2 $\pm$ 2.0	1.7 $\pm$ 0.4	1.7 $\pm$ 0.4	2.0 $\pm$ 0.4
B4.2	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	40.0 $\pm$ 4.4	7.0 $\pm$ 1.8	4.0 $\pm$ 1.4	1.0 $\pm$ 0.5	1.8 $\pm$ 0.5	1.3 $\pm$ 1.0
	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	NA	6.7	13.9	28.2	55.7	111.4
	Initial pH	8.0	7.9	7.9	7.9	7.9	7.9
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.080 $\pm$ 0.006	0.048 $\pm$ 0.006	0.034 $\pm$ 0.006	0.015 $\pm$ 0.001	0.014 $\pm$ 0.001	0.013 $\pm$ 0.001
B4.3	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	51.9 $\pm$ 4.5	27.1 $\pm$ 4.6	16.7 $\pm$ 4.2	2.0 $\pm$ 0.4	1.2 $\pm$ 0.5	1.0 $\pm$ 0.5
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	46.8 $\pm$ 2.8	24.0 $\pm$ 2.2	17.3 $\pm$ 2.2	1.0 $\pm$ 0.8	0.8 $\pm$ 0.5	0.8 $\pm$ 0.5
	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0004	5.8	11.9	23.2	47.2	94.5
	Initial pH	8.0	8.0	8.0	8.0	7.9	7.9
B4.4	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.068 $\pm$ 0.008	0.026 $\pm$ 0.003	0.015 $\pm$ 0.001	0.014 $\pm$ 0.001	0.014 $\pm$ 0.001	0.013 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	42.8 $\pm$ 5.8	10.9 $\pm$ 1.9	2.0 $\pm$ 0.4	1.7 $\pm$ 0.4	1.2 $\pm$ 0.5	1.0 $\pm$ 0.5
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	37.0 $\pm$ 4.9	9.0 $\pm$ 1.8	1.5 $\pm$ 0.6	1.0 $\pm$ 0.8	1.0 $\pm$ 0.8	1.0 $\pm$ 0.8
	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	NA	6.1	11	25.8	51.7	102.4
B4.5	Initial pH	8.1	8.1	8.0	8.0	8.1	8.0
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.046 $\pm$ 0.001	0.031 $\pm$ 0.003	0.022 $\pm$ 0.001	0.015 $\pm$ 0.001	0.015 $\pm$ 0.001	0.014 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	25.3 $\pm$ 0.9	14.1 $\pm$ 2.3	7.8 $\pm$ 0.9	2.5 $\pm$ 0.5	2.0 $\pm$ 0.4	1.7 $\pm$ 0.4
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	26.3 $\pm$ 1.7	15.0 $\pm$ 1.6	9.8 $\pm$ 1.7	1.8 $\pm$ 0.5	1.0 $\pm$ 0.8	0.8 $\pm$ 0.5

**Appendix 10 (4b) :** Raw data of cadmium toxicity tests with *Tetraselmis* sp., conducted in flasks, in the absence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	6.25	12.5	25.0	50.0	100.0
B4.5	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	NA	6.1	11	25.8	51.7	102.4
	Initial pH	8.1	8.1	8.0	8.0	8.1	8.0
	Final pH (n=3)	9.43 $\pm$ 0.10	8.65 $\pm$ 0.09	8.52 $\pm$ 0.07	8.17 $\pm$ 0.02	7.87 $\pm$ 0.02	7.83 $\pm$ 0.01
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.045 $\pm$ 0.004	0.026 $\pm$ 0.001	0.019 $\pm$ 0.001	0.016 $\pm$ 0.001	0.015 $\pm$ 0.001	0.014 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	24.8 $\pm$ 3.1	10.3 $\pm$ 0.5	5.0 $\pm$ 0.5	2.7 $\pm$ 0.5	2.5 $\pm$ 0.5	1.7 $\pm$ 0.4
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	21.0 $\pm$ 1.8	9.0 $\pm$ 1.8	5.5 $\pm$ 1.3	2.5 $\pm$ 0.6	1.8 $\pm$ 0.5	1.3 $\pm$ 0.5

Note :

# : Actual initial heavy metal concentration determined by ICP-AES

@ : OD determined by Multiskan MCC/340 MKII

\* : cell count X  $10^4$  cells.ml<sup>-1</sup>

a : estimated from  $OD_{550\text{nm}}$  by calculation using regression equation :  $y=761x-9.2$

b : determined by direct cell count of a randomly selected test sample from triplicate, using haemocytometer

Appendix 11 (1a) : Raw data of copper toxicity tests with *Chaetoceros calcitrans*, conducted in multiwell plates, in the presence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	1.0	1.8	3.2	5.6	10.0
C1.1	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0095	0.9	1.6	3.0	5.4	9.9
	Initial pH	8.0	8.1	8.1	8.0	8.0	8.0
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.209 $\pm$ 0.007	0.184 $\pm$ 0.007	0.166 $\pm$ 0.007	0.152 $\pm$ 0.004	0.145 $\pm$ 0.006	0.006 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	355.7 $\pm$ 12.2	311.8 $\pm$ 12.3	281.7 $\pm$ 11.8	256.8 $\pm$ 6.3	245.3 $\pm$ 9.6	4.1 $\pm$ 1.0
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	329.5 $\pm$ 18.3	318.0 $\pm$ 12.8	276.2 $\pm$ 9.1	264.5 $\pm$ 11.1	235.5 $\pm$ 12.9	3.8 $\pm$ 1.0
C1.2	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0092	0.8	1.5	2.4	4.5	8.1
	Initial pH	7.9	7.9	7.9	7.9	7.8	7.8
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.073 $\pm$ 0.006	0.105 $\pm$ 0.013	0.105 $\pm$ 0.012	0.088 $\pm$ 0.008	0.092 $\pm$ 0.009	0.006 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	120.3 $\pm$ 10.2	174.7 $\pm$ 22.1	174.7 $\pm$ 20.3	146.4 $\pm$ 13.9	152.7 $\pm$ 15.4	4.1 $\pm$ 1.0
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	115.3 $\pm$ 6.1	154.3 $\pm$ 3.0	177.3 $\pm$ 4.6	130.8 $\pm$ 5.9	130.5 $\pm$ 5.4	5.5 $\pm$ 1.3

Appendix 11 (1b) : Raw data of copper toxicity tests with *Chaetoceros calcitrans*, conducted in flasks, in the presence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	1.0	1.8	3.2	5.6	10.0
C1.3	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0092	0.8	1.5	2.4	4.5	8.1
	Initial pH	7.9	7.9	7.9	7.9	7.8	7.8
	Final pH (n=3)	9.46 $\pm$ 0.05	9.52 $\pm$ 0.03	9.50 $\pm$ 0.08	9.42 $\pm$ 9.28	7.28 $\pm$ 0.04	7.98 $\pm$ 0.17
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.094 $\pm$ 0.015	0.095 $\pm$ 0.001	0.089 $\pm$ 0.004	0.080 $\pm$ 0.003	0.071 $\pm$ 0.002	0.006 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	156.2 $\pm$ 25.2	157.9 $\pm$ 1.8	148.1 $\pm$ 6.6	131.9 $\pm$ 4.6	120.9 $\pm$ 4.0	4.1 $\pm$ 1.0
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	158.5 $\pm$ 5.7	166.8 $\pm$ 3.0	147.8 $\pm$ 3.0	135.3 $\pm$ 3.2	124.8 $\pm$ 3.7	5.3 $\pm$ 1.3

Note :

# : Actual initial heavy metal concentration determined by ICP-AES

@ : OD determined by Multiskan MCC/340 MKII

\* : cell count  $\times 10^4$  cells.mL<sup>-1</sup>

a : estimated from OD<sub>630nm</sub> by calculation using regression equation :  $y=1735x-6.9$

b : determined by direct cell count of a randomly selected test sample from triplicate, using haemocytometer

Appendix 11 (2a) : Raw data of copper toxicity tests with *Isochrysis galbana*, conducted in multiwell plates, in the presence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	1.0	1.8	3.2	5.6	10.0
C2.1	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0095	0.9	1.6	3.0	5.4	9.9
	Initial pH	8.0	8.1	8.1	8.0	8.0	8.0
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.083 $\pm$ 0.003	0.075 $\pm$ 0.002	0.070 $\pm$ 0.003	0.065 $\pm$ 0.003	0.066 $\pm$ 0.003	0.006 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	307.8 $\pm$ 12.8	278.5 $\pm$ 8.3	259.9 $\pm$ 10.0	238.6 $\pm$ 0.1	240.0 $\pm$ 10.1	3.2 $\pm$ 2.3
C2.2	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	298.0 $\pm$ 12.1	271.0 $\pm$ 11.6	268.0 $\pm$ 12.4	232.5 $\pm$ 10.8	223.0 $\pm$ 8.9	4.3 $\pm$ 0.5
	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0092	0.8	1.5	2.4	4.5	8.1
	Initial pH	7.9	7.9	7.9	7.9	7.8	7.8
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.036 $\pm$ 0.002	0.036 $\pm$ 0.001	0.038 $\pm$ 0.002	0.038 $\pm$ 0.001	0.038 $\pm$ 0.002	0.007 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	120.3 $\pm$ 8.3	120.3 $\pm$ 4.6	130.9 $\pm$ 6.2	128.3 $\pm$ 9.2	128.3 $\pm$ 6.1	6.9 $\pm$ 1.8
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	122.5 $\pm$ 5.5	122.5 $\pm$ 5.2	126.3 $\pm$ 2.8	116.0 $\pm$ 5.9	132.3 $\pm$ 8.0	1.5 $\pm$ 0.6

Appendix 11 (2b) : Raw data of copper toxicity tests with *Isochrysis galbana*, conducted in flasks, in the presence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	1.0	1.8	3.2	5.6	10.0
C2.3	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0092	0.8	1.5	2.4	4.5	8.1
	Initial pH	7.9	7.9	7.9	7.9	7.8	7.8
	Final pH (n=3)	8.93 $\pm$ 0.03	8.95 $\pm$ 0.04	8.99 $\pm$ 0.04	9.00 $\pm$ 0.03	8.98 $\pm$ 0.03	8.00 $\pm$ 0.06
	96h OD $\pm$ SD (n=3) <sup>*</sup>	0.049 $\pm$ 0.002	0.053 $\pm$ 0.004	0.056 $\pm$ 0.005	0.055 $\pm$ 0.003	0.050 $\pm$ 0.003	0.007 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	174.8 $\pm$ 6.1	188.1 $\pm$ 15.1	201.4 $\pm$ 18.3	198.7 $\pm$ 10.1	178.9 $\pm$ 10.2	7.2 $\pm$ 2.3
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	171.8 $\pm$ 5.9	202.5 $\pm$ 6.5	185.5 $\pm$ 4.8	208.5 $\pm$ 3.5	175.0 $\pm$ 8.4	2.5 $\pm$ 0.6

Note :

# : Actual initial heavy metal concentration determined by ICP-AES

@ : OD determined by Multiskan MCC/340 MKII

\* : cell count  $\times 10^4$  cells. $\text{ml}^{-1}$

a : estimated from OD<sub>450nm</sub> by calculation using regression equation :  $y=3989.4x-22.0$

b : determined by direct cell count of a randomly selected test sample from triplicate, using haemocytometer

Appendix 11 (3a) : Raw data of copper toxicity tests with *Tetraselmis tetrahele*, conducted in multiwell plates, in the presence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	1.0	1.8	3.2	5.6	10.0
C3.1	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0081	0.8	1.5	2.4	4.5	8.1
	Initial pH	7.9	7.9	7.9	7.9	7.8	7.8
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.162 $\pm$ 0.004	0.143 $\pm$ 0.017	0.130 $\pm$ 0.012	0.142 $\pm$ 0.016	0.138 $\pm$ 0.020	0.031 $\pm$ 0.005
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	101.3 $\pm$ 2.6	89.0 $\pm$ 11.2	80.6 $\pm$ 7.7	88.4 $\pm$ 10.4	85.3 $\pm$ 13.1	16.5 $\pm$ 3.2
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	97.5 $\pm$ 8.2	84.8 $\pm$ 5.7	74.0 $\pm$ 3.7	101.5 $\pm$ 6.5	73.0 $\pm$ 2.2	14.5 $\pm$ 2.1

Appendix 11 (3b) : Raw data of copper toxicity tests with *Tetraselmis tetrahele*, conducted in flasks, in the presence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	1.0	1.8	3.2	5.6	10.0
C3.2	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	NA	NA	NA	NA	NA	NA
	Initial pH	8.0	8.0	8.0	8.0	8.0	7.9
	Final pH (n=3)	9.57 $\pm$ 0.02	9.53 $\pm$ 0.02	9.60 $\pm$ 0.04	9.58 $\pm$ 0.04	9.42 $\pm$ 0.02	8.02 $\pm$ 0.02
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.057 $\pm$ 0.002	0.052 $\pm$ 0.002	0.050 $\pm$ 0.001	0.045 $\pm$ 0.004	0.033 $\pm$ 0.004	0.011 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	32.9 $\pm$ 1.0	29.7 $\pm$ 1.0	28.4 $\pm$ 0.4	25.1 $\pm$ 2.3	17.8 $\pm$ 2.4	3.1 $\pm$ 0.3
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	33.5 $\pm$ 2.4	29.3 $\pm$ 3.3	28.0 $\pm$ 4.2	26.8 $\pm$ 2.8	18.3 $\pm$ 1.7	3.5 $\pm$ 1.3

Note :

# : Actual initial heavy metal concentration determined by ICP-AES (NA=not available)

@ : OD determined by Multiskan MCC/340 MKII

\* : cell count X 10<sup>6</sup> cells.ml<sup>-1</sup>

a : estimated from OD<sub>600nm</sub> by calculation using regression equation : y=647.4x-3.8

b : determined by direct cell count of a randomly selected test sample from triplicate, using haemocytometer

## Appendix 11(4)

Appendix 11 (4a) : Raw data of copper toxicity tests with *Tetraselmis* sp., conducted in multiwell plates, in the presence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	1.0	1.8	3.2	5.6	10.0
C4.1	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0081	0.9	1.6	3.0	5.2	8.8
	Initial pH	8.0	8.0	7.9	7.9	7.9	7.6
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.083 $\pm$ 0.003	0.055 $\pm$ 0.005	0.055 $\pm$ 0.009	0.044 $\pm$ 0.005	0.054 $\pm$ 0.010	0.013 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	54.2 $\pm$ 1.9	32.9 $\pm$ 3.8	32.7 $\pm$ 6.8	24.3 $\pm$ 4.0	32.2 $\pm$ 7.7	1.0 $\pm$ 0.5
C4.2	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	49.8 $\pm$ 4.2	31.8 $\pm$ 2.8	25.8 $\pm$ 1.5	18.3 $\pm$ 3.3	28.0 $\pm$ 3.6	0.8 $\pm$ 0.5
	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0095	0.9	1.6	3.0	5.4	9.9
	Initial pH	8.0	8.1	8.1	8.0	8.0	8.0
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.198 $\pm$ 0.006	0.142 $\pm$ 0.013	0.138 $\pm$ 0.013	0.131 $\pm$ 0.018	0.130 $\pm$ 0.008	0.015 $\pm$ 0.002
C4.3	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	141.2 $\pm$ 4.4	98.9 $\pm$ 10.1	96.1 $\pm$ 9.9	90.3 $\pm$ 13.3	89.5 $\pm$ 5.7	2.5 $\pm$ 1.1
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	143.0 $\pm$ 3.5	102.0 $\pm$ 5.0	101.8 $\pm$ 7.9	78.0 $\pm$ 3.7	83.0 $\pm$ 2.2	2.8 $\pm$ 1.0
	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0092	0.8	1.5	2.4	4.5	8.1
	Initial pH	7.9	7.9	7.9	7.9	7.8	7.8
C4.4	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.159 $\pm$ 0.020	0.106 $\pm$ 0.011	0.108 $\pm$ 0.003	0.110 $\pm$ 0.004	0.106 $\pm$ 0.003	0.047 $\pm$ 0.002
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	111.5 $\pm$ 14.9	71.7 $\pm$ 8.7	73.0 $\pm$ 2.3	74.8 $\pm$ 2.9	71.2 $\pm$ 2.3	26.5 $\pm$ 1.3
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	112.8 $\pm$ 3.3	70.5 $\pm$ 3.1	70.3 $\pm$ 4.0	70.5 $\pm$ 4.2	70.0 $\pm$ 3.1	25.5 $\pm$ 1.9

Appendix 11 (4b) : Raw data of copper toxicity tests with *Tetraselmis* sp., conducted in flasks, in the presence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	1.0	1.8	3.2	5.6	10.0
C4.4	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	NA	NA	NA	NA	NA	NA
	Initial pH	8.0	8.0	8.0	8.0	8.0	7.9
	Final pH (n=3)	9.49 $\pm$ 0.02	9.42 $\pm$ 0.02	9.40 $\pm$ 0.02	9.39 $\pm$ 0.04	9.32 $\pm$ 0.01	8.44 $\pm$ 0.69
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.075 $\pm$ 0.004	0.070 $\pm$ 0.004	0.062 $\pm$ 0.003	0.062 $\pm$ 0.003	0.050 $\pm$ 0.002	0.013 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	48.1 $\pm$ 3.1	44.1 $\pm$ 2.7	39.5 $\pm$ 1.3	37.8 $\pm$ 2.2	29.0 $\pm$ 1.6	1.0 $\pm$ 0.5
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	52.3 $\pm$ 1.7	45.8 $\pm$ 1.7	39.3 $\pm$ 1.8	39.8 $\pm$ 4.0	26.0 $\pm$ 1.6	1.3 $\pm$ 0.5

Note :

# : Actual initial heavy metal concentration determined by ICP-AES (NA=not available)

@ : OD determined by Multiskan MCC/340 MKII

\* : cell count X  $10^4$  cells.ml<sup>-1</sup>

a : estimated from OD<sub>630nm</sub> by calculation using regression equation :  $y=761x-9.2$

b : determined by direct cell count of a randomly selected test sample from triplicate, using haemocytometer

## Appendix 12(1)

**Appendix 12 (1a) :** Raw data of copper toxicity tests with *Chaetoceros calcitrans*, conducted in multiwell plates, in the absence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	0.10	0.18	0.32	0.56	1.00
D1.1	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	NA	0.02	0.10	0.20	0.40	0.80
	Initial pH	8.0	7.9	7.9	7.9	7.9	7.9
	96h OD $\pm$ SD (n=3) @	0.070 $\pm$ 0.001	0.049 $\pm$ 0.004	0.020 $\pm$ 0.003	0.006 $\pm$ 0.001	0.005 $\pm$ 0.001	0.005 $\pm$ 0.001
	Estimated* Cell Count $\pm$ SD* (n=3)	115.2 $\pm$ 1.0	81.0 $\pm$ 3.6	27.8 $\pm$ 6.0	2.9 $\pm$ 1.0	2.4 $\pm$ 1.0	2.4 $\pm$ 1.0
D1.2	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	113.0 $\pm$ 5.0	78.5 $\pm$ 6.0	32.5 $\pm$ 2.5	3.5 $\pm$ 1.3	2.3 $\pm$ 0.5	1.8 $\pm$ 0.05
	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0079	0.13	0.20	0.31	0.52	0.86
	Initial pH	8.3	8.1	8.2	8.2	8.2	8.2
	96h OD $\pm$ SD (n=3) @	0.096 $\pm$ 0.006	0.018 $\pm$ 0.001	0.018 $\pm$ 0.001	0.008 $\pm$ 0.001	0.006 $\pm$ 0.001	0.005 $\pm$ 0.001
	Estimated* Cell Count $\pm$ SD* (n=3)	160.3 $\pm$ 9.5	24.9 $\pm$ 1.0	23.8 $\pm$ 1.0	6.4 $\pm$ 1.0	4.1 $\pm$ 1.0	2.4 $\pm$ 1.0
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	149.0 $\pm$ 5.7	22.3 $\pm$ 2.2	23.3 $\pm$ 2.2	5.8 $\pm$ 1.5	5.0 $\pm$ 0.8	2.3 $\pm$ 1.3

**Appendix 12 (1b) :** Raw data of copper toxicity tests with *Chaetoceros calcitrans*, conducted in flasks, in the absence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	0.10	0.18	0.32	0.56	1.00
D1.3	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0079	0.13	0.20	0.31	0.52	0.86
	Initial pH	8.3	8.1	8.2	8.2	8.2	8.2
	Final pH (n=3)	9.60 $\pm$ 0.05	8.28 $\pm$ 0.03	8.68 $\pm$ 0.11	8.04 $\pm$ 0.02	8.04 $\pm$ 0.02	7.99 $\pm$ 0.01
	96h OD $\pm$ SD (n=3) @	0.082 $\pm$ 0.006	0.006 $\pm$ 0.001	0.006 $\pm$ 0.001	0.006 $\pm$ 0.001	0.005 $\pm$ 0.001	0.005 $\pm$ 0.001
	Estimated* Cell Count $\pm$ SD* (n=3)	134.8 $\pm$ 10.2	4.1 $\pm$ 1.0	2.9 $\pm$ 1.0	2.9 $\pm$ 1.0	2.4 $\pm$ 1.0	2.4 $\pm$ 1.0
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	129.0 $\pm$ 4.7	2.5 $\pm$ 0.6	2.8 $\pm$ 1.0	4.3 $\pm$ 1.0	2.5 $\pm$ 0.7	1.0 $\pm$ 0.8

Note :

# : Actual initial heavy metal concentration determined by ICP-AES

@ : OD determined by Multiskan MCC/340 MKII

\* : cell count  $\times 10^4$  cells.mL $^{-1}$

a : estimated from OD<sub>250nm</sub> by calculation using regression equation :  $y=1735x-6.9$

b : determined by direct cell count of a randomly selected test sample from triplicate, using haemocytometer

Appendix 12 (2) : Raw data of copper toxicity tests with *Isochrysis galbana*, conducted in multiwell plates, in the absence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	0.10	0.18	0.32	0.56	1.00
D2.1	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	NA	0.02	0.10	0.20	0.40	0.80
	Initial pH	8.0	7.9	7.9	7.9	7.9	7.9
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.035 $\pm$ 0.001	0.023 $\pm$ 0.004	0.007 $\pm$ 0.007	0.007 $\pm$ 0.001	0.006 $\pm$ 0.001	0.006 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	117.6 $\pm$ 4.0	69.8 $\pm$ 17.4	4.6 $\pm$ 2.3	4.6 $\pm$ 2.3	3.2 $\pm$ 2.3	3.2 $\pm$ 2.3
D2.2	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	122.8 $\pm$ 5.3	62.5 $\pm$ 4.2	1.8 $\pm$ 1.0	0.8 $\pm$ 0.5	0.8 $\pm$ 0.5	0.8 $\pm$ 0.5
	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0078	0.07	0.15	0.28	0.60	1.20
	Initial pH	8.0	7.9	7.9	7.8	7.8	7.7
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.055 $\pm$ 0.003	0.008 $\pm$ 0.001	0.007 $\pm$ 0.001	0.007 $\pm$ 0.001	0.006 $\pm$ 0.001	0.006 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	197.4 $\pm$ 12.0	8.6 $\pm$ 2.3	7.2 $\pm$ 2.3	4.6 $\pm$ 2.3	3.2 $\pm$ 2.3	3.2 $\pm$ 2.3
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	216.0 $\pm$ 2.9	8.8 $\pm$ 2.8	6.0 $\pm$ 0.8	3.0 $\pm$ 0.8	1.0 $\pm$ 0.8	1.0 $\pm$ 0.8

Note :  
<sup>#</sup> : Actual initial heavy metal concentration determined by ICP-AES

@ : OD determined by Multiskan MCC/340 MKII

\* : cell count  $\times 10^6$  cells. $\text{mL}^{-1}$

a : estimated from OD<sub>96h</sub> by calculation using regression equation :  $y=3989.4x-22.0$

b : determined by direct cell count of a randomly selected test sample from triplicate, using haemocytometer

Appendix 12 (3a) : Raw data of copper toxicity tests with *Tetraselmis tetrahele*, conducted in multiwell plates, in the absence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	0.10	0.18	0.32	0.56	1.00
D3.1	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	NA	0.02	0.10	0.20	0.40	0.80
	Initial pH	8.0	7.9	7.9	7.9	7.9	7.9
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.053 $\pm$ 0.005	0.039 $\pm$ 0.003	0.032 $\pm$ 0.006	0.020 $\pm$ 0.004	0.010 $\pm$ 0.001	0.009 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	30.5 $\pm$ 2.9	21.4 $\pm$ 2.0	16.9 $\pm$ 3.6	9.4 $\pm$ 3.7	2.5 $\pm$ 0.4	1.8 $\pm$ 0.4
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	37.0 $\pm$ 3.6	18.0 $\pm$ 2.2	14.3 $\pm$ 1.7	10.0 $\pm$ 1.8	3.8 $\pm$ 1.0	2.3 $\pm$ 1.0

Appendix 12 (3b) : Raw data of copper toxicity tests with *Tetraselmis tetrahele*, conducted in flasks, in the absence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	0.10	0.18	0.32	0.56	1.00
D3.2	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0079	0.13	0.20	0.31	0.52	0.86
	Initial pH	8.3	8.1	8.2	8.2	8.2	8.2
	Final pH (n=3)	9.51 $\pm$ 0.10	8.90 $\pm$ 0.07	9.01 $\pm$ 0.08	8.24 $\pm$ 0.03	8.24 $\pm$ 0.03	8.06 $\pm$ 0.02
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.067 $\pm$ 0.009	0.035 $\pm$ 0.001	0.034 $\pm$ 0.001	0.017 $\pm$ 0.001	0.017 $\pm$ 0.001	0.016 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	39.6 $\pm$ 5.9	19.1 $\pm$ 0.3	18.4 $\pm$ 0.4	7.0 $\pm$ 0.3	7.0 $\pm$ 0.3	6.8 $\pm$ 0.3
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	38.3 $\pm$ 2.6	16.8 $\pm$ 2.2	16.8 $\pm$ 1.0	7.8 $\pm$ 1.7	5.8 $\pm$ 1.0	6.8 $\pm$ 1.0

Note :

# : Actual initial heavy metal concentration determined by ICP-AES

@ : OD determined by Multiskan MCC/340 MKII

\* : cell count  $\times 10^6$  cells. $\text{mL}^{-1}$

a : estimated from OD<sub>625nm</sub> by calculation using regression equation :  $y=647.4x-3.8$

b : determined by direct cell count of a randomly selected test sample from triplicate, using haemocytometer

Appendix 12 (4) : Raw data of copper toxicity tests with *Tetraselmis* sp., conducted in multiwell plates, in the absence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	0.10	0.18	0.32	0.56	1.00
D4.1	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	NA	0.1	0.2	0.3	0.5	0.8
	Initial pH	8.1	7.9	7.9	8.0	8.0	7.9
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.080 $\pm$ 0.005	0.079 $\pm$ 0.001	0.077 $\pm$ 0.003	0.059 $\pm$ 0.004	0.024 $\pm$ 0.002	0.015 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	51.9 $\pm$ 3.8	51.0 $\pm$ 0.7	49.2 $\pm$ 2.2	35.7 $\pm$ 3.0	9.3 $\pm$ 1.7	2.5 $\pm$ 0.5
D4.2	Actual <sup>b</sup> Cell Count $\pm$ SD*(n=4)	54.8 $\pm$ 3.0	51.0 $\pm$ 2.6	46.8 $\pm$ 3.8	35.0 $\pm$ 3.4	8.0 $\pm$ 1.8	2.0 $\pm$ 0.8
	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0078	0.07	0.15	0.28	0.60	1.20
	Initial pH	8.0	7.9	7.9	7.8	7.8	7.7
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.100 $\pm$ 0.005	0.086 $\pm$ 0.004	0.074 $\pm$ 0.001	0.065 $\pm$ 0.002	0.022 $\pm$ 0.001	0.018 $\pm$ 0.001
D4.3	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	66.9 $\pm$ 1.3	56.5 $\pm$ 8.8	47.1 $\pm$ 0.8	40.2 $\pm$ 7.7	7.8 $\pm$ 1.7	4.8 $\pm$ 0.5
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	65.5 $\pm$ 4.0	58.8 $\pm$ 2.0	45.8 $\pm$ 3.3	36.5 $\pm$ 0.5	7.3 $\pm$ 0.2	4.5 $\pm$ 1.0
	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0079	0.13	0.20	0.31	0.52	0.86
	Initial pH	8.3	8.1	8.2	8.2	8.2	8.2
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.090 $\pm$ 0.007	0.057 $\pm$ 0.001	0.057 $\pm$ 0.001	0.057 $\pm$ 0.005	0.044 $\pm$ 0.002	0.037 $\pm$ 0.003
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	59.3 $\pm$ 5.0	34.2 $\pm$ 0.8	34.4 $\pm$ 0.4	33.9 $\pm$ 3.5	24.5 $\pm$ 1.2	18.9 $\pm$ 2.0
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	63.0 $\pm$ 2.4	35.0 $\pm$ 2.2	34.5 $\pm$ 3.0	31.8 $\pm$ 2.1	24.0 $\pm$ 3.7	18.0 $\pm$ 1.6

Note :  
# : Actual initial heavy metal concentration determined by ICP-AES  
@ : OD determined by Multiskan MCC/340 MKII

\* : cell count  $\times 10^4$  cells  $\text{mL}^{-1}$

a : estimated from  $OD_{\text{control}}$  by calculation using regression equation :  $y = 761x - 9.2$

b : determined by direct cell count of a randomly selected test sample from triplicate, using haemocytometer

Appendix 13 (1) : Raw data of manganese toxicity tests with *Chaetoceros calcitrans*, conducted in multiwell plates, in the presence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	6.25	12.5	25.0	50.0	100.0
E1.1	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0888	5.7	12.0	23.7	46.8	95.4
	Initial pH	8.0	8.0	8.0	8.0	8.0	8.0
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.200 $\pm$ 0.006	0.175 $\pm$ 0.003	0.169 $\pm$ 0.015	0.164 $\pm$ 0.009	0.125 $\pm$ 0.010	0.052 $\pm$ 0.003
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	340.1 $\pm$ 10.8	296.1 $\pm$ 4.3	286.3 $\pm$ 26.7	278.2 $\pm$ 16.1	210.0 $\pm$ 16.6	83.9 $\pm$ 5.3
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	339.8 $\pm$ 7.5	301.3 $\pm$ 18.3	274.0 $\pm$ 10.7	274.5 $\pm$ 13.9	233.8 $\pm$ 9.5	88.0 $\pm$ 8.5
E1.2	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0905	5.8	11.5	23.0	45.9	89.2
	Initial pH	8.1	8.2	8.1	8.1	8.0	8.0
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.213 $\pm$ 0.010	0.199 $\pm$ 0.012	0.198 $\pm$ 0.021	0.179 $\pm$ 0.016	0.131 $\pm$ 0.003	0.071 $\pm$ 0.011
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	363.2 $\pm$ 16.5	337.8 $\pm$ 20.0	336.1 $\pm$ 35.7	304.2 $\pm$ 28.5	238.3 $\pm$ 35.6	115.7 $\pm$ 19.4
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	350.5 $\pm$ 6.0	305.0 $\pm$ 4.5	303.8 $\pm$ 6.3	287.0 $\pm$ 6.7	209.5 $\pm$ 4.1	96.8 $\pm$ 4.3
E1.3	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0915	4.5	12.2	20.6	49.6	94.3
	Initial pH	8.0	8.1	8.0	8.1	8.2	8.0
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.153 $\pm$ 0.010	0.150 $\pm$ 0.004	0.133 $\pm$ 0.003	0.137 $\pm$ 0.001	0.108 $\pm$ 0.006	0.051 $\pm$ 0.002
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	258.5 $\pm$ 16.6	254.5 $\pm$ 7.0	223.8 $\pm$ 9.2	230.8 $\pm$ 1.7	179.9 $\pm$ 9.6	81.6 $\pm$ 3.0
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	234.8 $\pm$ 3.7	256.8 $\pm$ 5.9	208.5 $\pm$ 10.6	234.8 $\pm$ 3.9	175.5 $\pm$ 4.4	81.5 $\pm$ 1.9

Note :  
# : Actual initial heavy metal concentration determined by ICP-AES  
@ : OD determined by Multiskan MCC/340 MKII

\* : cell count  $\times 10^4$  cells. $\text{mL}^{-1}$

a : estimated from OD<sub>400nm</sub> by calculation using regression equation :  $y=1735x-6.9$

b : determined by direct cell count of a randomly selected test sample from triplicate, using haemocytometer

Appendix 13(2) : Raw data of manganese toxicity tests with *Isochrysis galbana*, conducted in multiwell plates, in the presence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	6.25	12.5	25.0	50.0	100.0
E2.1	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0888	5.7	12.0	23.7	46.8	95.4
	Initial pH	8.0	8.0	8.0	8.0	8.0	8.0
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.103 $\pm$ 0.010	0.084 $\pm$ 0.012	0.086 $\pm$ 0.008	0.060 $\pm$ 0.004	0.040 $\pm$ 0.006	0.023 $\pm$ 0.002
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	387.6 $\pm$ 39.3	311.8 $\pm$ 47.6	319.8 $\pm$ 30.2	218.7 $\pm$ 14.0	143.6 $\pm$ 38.3	71.1 $\pm$ 6.0
E2.2	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	361.0 $\pm$ 15.3	309.5 $\pm$ 15.3	342.3 $\pm$ 9.0	211.8 $\pm$ 3.6	124.8 $\pm$ 10.5	78.8 $\pm$ 9.2
	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0905	5.8	11.5	23.0	46.0	89.2
	Initial pH	8.1	8.2	8.1	8.1	8.0	8.0
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.077 $\pm$ 0.002	0.069 $\pm$ 0.007	0.058 $\pm$ 0.006	0.064 $\pm$ 0.002	0.040 $\pm$ 0.001	0.017 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	285.2 $\pm$ 6.9	253.3 $\pm$ 26.1	208.1 $\pm$ 23.0	232.0 $\pm$ 8.3	138.9 $\pm$ 2.3	44.5 $\pm$ 2.3
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	280.3 $\pm$ 10.8	254.5 $\pm$ 7.3	184.5 $\pm$ 4.2	244.0 $\pm$ 5.0	138.5 $\pm$ 8.2	35.5 $\pm$ 2.9

Note :

# : Actual initial heavy metal concentration determined by ICP-AES

@ : OD determined by Multiskan MCC/340 MKII

\* : cell count  $\times 10^6$  cells.mL<sup>-1</sup>

a : estimated from OD<sub>96h</sub> by calculation using regression equation :  $y = 3989.4x - 22.0$

b : determined by direct cell count of a randomly selected test sample from triplicate, using haemocytometer

Appendix 13(3a) : Raw data of manganese toxicity tests with *Tetraselmis tetrahele*, conducted in multiwell plates, in the presence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	6.25	12.5	25.0	50.0	100.0
E3.1	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0915	4.5	12.20	20.60	49.60	94.30
	Initial pH	8.0	8.0	8.0	8.0	8.0	8.0
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.172 $\pm$ 0.011	0.151 $\pm$ 0.008	0.117 $\pm$ 0.016	0.123 $\pm$ 0.009	0.125 $\pm$ 0.008	0.061 $\pm$ 0.003
	Estimated* Cell Count $\pm$ SD* (n=3)	107.5 $\pm$ 7.2	94.0 $\pm$ 5.3	72.1 $\pm$ 11.1	76.1 $\pm$ 6.0	77.1 $\pm$ 5.1	35.5 $\pm$ 1.7
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	100.0 $\pm$ 12.4	98.8 $\pm$ 8.0	69.5 $\pm$ 4.2	80.0 $\pm$ 7.3	75.5 $\pm$ 4.8	37.5 $\pm$ 2.1

Appendix 13(3b) : Raw data of manganese toxicity tests with *Tetraselmis tetrahele*, conducted in flasks, in the presence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	6.25	12.5	25.0	50.0	100.0
E3.2	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0915	4.5	12.2	20.6	49.6	94.3
	Initial pH	8.0	8.1	8.0	8.1	8.2	8.0
	Final pH (n=3)	9.54 $\pm$ 0.02	9.38 $\pm$ 0.03	9.15 $\pm$ 0.02	8.94 $\pm$ 0.02	8.69 $\pm$ 0.05	8.34 $\pm$ 0.04
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.061 $\pm$ 0.009	0.062 $\pm$ 0.001	0.052 $\pm$ 0.001	0.061 $\pm$ 0.003	0.049 $\pm$ 0.002	0.030 $\pm$ 0.002
	Estimated* Cell Count $\pm$ SD* (n=3)	35.9 $\pm$ 6.0	36.1 $\pm$ 0.8	29.6 $\pm$ 0.8	35.7 $\pm$ 2.3	28.1 $\pm$ 1.0	15.9 $\pm$ 1.5
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	42.0 $\pm$ 2.8	34.5 $\pm$ 3.3	29.5 $\pm$ 4.2	33.5 $\pm$ 2.1	27.8 $\pm$ 3.2	15.0 $\pm$ 0.8

Note :

# : Actual initial heavy metal concentration determined by ICP-AES

@ : OD determined by Multiskan MCC/340 MKII

\* : cell count X  $10^6$  cells.mL<sup>-1</sup>

a : estimated from OD<sub>650nm</sub> by calculation using regression equation :  $y=647.4x-3.8$

b : determined by direct cell count of a randomly selected test sample from triplicate, using haemocytometer

Appendix 13(4a) : Raw data of manganese toxicity tests with *Tetraselmis* sp., conducted in multiwell plates, in the presence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	6.25	12.5	25.0	50.0	100.0
E4.1	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0905	5.8	11.5	23.0	45.9	89.2
	Initial pH	8.1	8.2	8.1	8.1	8.0	8.0
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.094 $\pm$ 0.017	0.094 $\pm$ 0.018	0.087 $\pm$ 0.020	0.079 $\pm$ 0.010	0.077 $\pm$ 0.010	0.048 $\pm$ 0.007
	Estimated <sup>a</sup> Cell Count $\pm$ SD <sup>a</sup> (n=3)	62.1 $\pm$ 12.8	62.1 $\pm$ 13.7	56.7 $\pm$ 15.1	50.9 $\pm$ 8.0	49.7 $\pm$ 8.4	27.3 $\pm$ 5.3
E4.2	Actual <sup>b</sup> Cell Count $\pm$ SD <sup>b</sup> (n=4)	73.5 $\pm$ 3.9	61.3 $\pm$ 2.2	43.8 $\pm$ 3.0	49.5 $\pm$ 4.1	45.0 $\pm$ 2.9	34.5 $\pm$ 2.4
	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0915	4.5	12.2	20.6	49.6	94.3
	Initial pH	8.0	8.1	8.0	8.1	8.2	8.0
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.210 $\pm$ 0.006	0.191 $\pm$ 0.006	0.136 $\pm$ 0.003	0.124 $\pm$ 0.001	0.119 $\pm$ 0.002	0.059 $\pm$ 0.002
	Estimated <sup>a</sup> Cell Count $\pm$ SD <sup>a</sup> (n=3)	150.6 $\pm$ 4.6	136.4 $\pm$ 4.7	94.0 $\pm$ 1.9	84.9 $\pm$ 0.9	81.1 $\pm$ 1.2	35.7 $\pm$ 1.3
	Actual <sup>b</sup> Cell Count $\pm$ SD <sup>b</sup> (n=4)	150.8 $\pm$ 2.5	131.3 $\pm$ 4.3	93.0 $\pm$ 3.2	84.8 $\pm$ 2.8	83.3 $\pm$ 3.3	31.5 $\pm$ 3.7

Appendix 13(4b) : Raw data of manganese toxicity tests with *Tetraselmis* sp., conducted in flasks, in the presence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	6.25	12.5	25.0	50.0	100.0
E4.3	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0915	4.5	12.2	20.6	49.6	94.3
	Initial pH	8.0	8.1	8.0	8.1	8.2	8.0
	Final pH (n=3)	9.54 $\pm$ 0.04	9.36 $\pm$ 0.02	9.15 $\pm$ 0.03	8.91 $\pm$ 0.01	8.56 $\pm$ 0.04	8.31 $\pm$ 0.02
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.060 $\pm$ 0.001	0.062 $\pm$ 0.002	0.046 $\pm$ 0.001	0.053 $\pm$ 0.001	0.045 $\pm$ 0.002	0.027 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD <sup>a</sup> (n=3)	36.2 $\pm$ 0.5	38.2 $\pm$ 1.6	26.1 $\pm$ 0.5	31.4 $\pm$ 0.9	25.3 $\pm$ 1.6	11.1 $\pm$ 0.9
	Actual <sup>b</sup> Cell Count $\pm$ SD <sup>b</sup> (n=4)	34.8 $\pm$ 3.5	40.3 $\pm$ 2.4	25.0 $\pm$ 2.6	32.0 $\pm$ 3.2	24.0 $\pm$ 2.9	9.5 $\pm$ 2.4

Note :

# : Actual initial heavy metal concentration determined by ICP-AES

@ : OD determined by Multiskan MCC/340 MKII

\* : cell count X  $10^4$  cells. $\text{mL}^{-1}$

a : estimated from OD<sub>630nm</sub> by calculation using regression equation :  $y=761x-9.2$

b : determined by direct cell count of a randomly selected test sample from triplicate, using haemocytometer

## Appendix 14(1)

**Appendix 14 (1a) :** Raw data of manganese toxicity tests with *Chaetoceros calcitrans*, conducted in multiwell plates, in the absence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	6.25	12.5	25.0	50.0	100.0
F1.1	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0955	5.7	11.5	22.9	46.1	92.3
	Initial pH	8.0	8.0	8.0	7.9	7.9	7.9
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.150 $\pm$ 0.009	0.105 $\pm$ 0.003	0.100 $\pm$ 0.012	0.063 $\pm$ 0.002	0.052 $\pm$ 0.003	0.031 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	253.3 $\pm$ 14.8	174.7 $\pm$ 5.3	166.6 $\pm$ 20.0	102.4 $\pm$ 3.0	82.7 $\pm$ 5.3	46.9 $\pm$ 1.7
	Actual <sup>b</sup> Cell Count $\pm$ SD * (n=4)	253.5 $\pm$ 7.2	167.8 $\pm$ 4.7	149.0 $\pm$ 5.4	97.5 $\pm$ 5.1	76.3 $\pm$ 2.6	43.0 $\pm$ 4.2

**Appendix 14 (1b) :** Raw data of manganese toxicity tests with *Chaetoceros calcitrans*, conducted in flasks, in the absence of EDTA

Exp No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	6.25	12.5	25.0	50.0	100.0
F1.2	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0920	4.9	11.9	24.0	48.6	97.9
	Initial pH	8.2	8.2	8.1	8.1	8.2	8.2
	Final pH (n=3)	9.34 $\pm$ 0.08	8.94 $\pm$ 0.05	8.59 $\pm$ 0.01	8.33 $\pm$ 0.04	8.12 $\pm$ 0.05	7.92 $\pm$ 0.01
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.058 $\pm$ 0.002	0.048 $\pm$ 0.004	0.037 $\pm$ 0.004	0.027 $\pm$ 0.002	0.018 $\pm$ 0.004	0.017 $\pm$ 0.004
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	94.3 $\pm$ 2.7	76.4 $\pm$ 6.3	57.3 $\pm$ 6.2	40.5 $\pm$ 2.7	24.3 $\pm$ 6.2	22.0 $\pm$ 6.6
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	97.3 $\pm$ 4.3	74.5 $\pm$ 2.6	51.5 $\pm$ 5.2	41.8 $\pm$ 5.2	30.5 $\pm$ 3.1	21.0 $\pm$ 2.9

Note :

# : Actual initial heavy metal concentration determined by ICP-AES

@ : OD determined by Multiskan MCC/340 MKII

\* : cell count X  $10^4$  cells. $\text{mL}^{-1}$

a : estimated from OD<sub>620nm</sub> by calculation using regression equation :  $y=1735x-6.9$

b : determined by direct cell count of a randomly selected test sample from triplicate, using haemocytometer

Appendix 14 (2a) : Raw data of manganese toxicity tests with *Isochrysis galbana*, conducted in multiwell plates, in the absence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	6.25	12.5	25.0	50.0	100.0
F2.1	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0924	5.9	11.8	23.2	46.0	97.7
	Initial pH	8.0	8.0	8.0	8.0	8.0	8.0
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.038 $\pm$ 0.002	0.021 $\pm$ 0.002	0.014 $\pm$ 0.001	0.010 $\pm$ 0.001	0.007 $\pm$ 0.001	0.007 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	129.6 $\pm$ 6.9	60.5 $\pm$ 6.1	35.2 $\pm$ 2.3	16.6 $\pm$ 2.3	7.2 $\pm$ 2.3	7.2 $\pm$ 2.3
F2.2	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	136.0 $\pm$ 3.9	52.8 $\pm$ 2.9	36.5 $\pm$ 3.5	18.0 $\pm$ 2.9	5.8 $\pm$ 1.0	6.0 $\pm$ 1.8
	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0920	4.9	11.9	24.0	48.6	97.9
	Initial pH	8.2	8.2	8.1	8.1	8.2	8.2
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.029 $\pm$ 0.003	0.018 $\pm$ 0.001	0.017 $\pm$ 0.002	0.014 $\pm$ 0.002	0.013 $\pm$ 0.001	0.008 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	93.7 $\pm$ 13.9	48.5 $\pm$ 2.3	45.8 $\pm$ 6.9	35.2 $\pm$ 6.1	28.6 $\pm$ 2.3	11.2 $\pm$ 2.3
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	108.0 $\pm$ 3.7	49.5 $\pm$ 4.3	51.3 $\pm$ 3.0	41.5 $\pm$ 4.7	30.0 $\pm$ 2.6	6.5 $\pm$ 1.3

Appendix 14 (2b) : Raw data of manganese toxicity tests with *Isochrysis galbana*, conducted in flasks, in the absence of EDTA

Exp No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	6.25	12.5	25.0	50.0	100.0
F2.3	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0920	4.9	11.9	24.0	48.6	97.9
	Initial pH	8.2	8.2	8.1	8.1	8.2	8.2
	Final pH (n=3)	8.51 $\pm$ 0.05	8.23 $\pm$ 0.10	8.18 $\pm$ 0.07	7.98 $\pm$ 0.02	7.91 $\pm$ 0.02	7.89 $\pm$ 0.02
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.026 $\pm$ 0.001	0.016 $\pm$ 0.003	0.014 $\pm$ 0.001	0.010 $\pm$ 0.001	0.008 $\pm$ 0.001	0.007 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	80.4 $\pm$ 2.3	43.2 $\pm$ 11.5	32.6 $\pm$ 2.3	19.2 $\pm$ 2.3	8.6 $\pm$ 2.3	7.2 $\pm$ 2.3
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	80.5 $\pm$ 5.6	47.0 $\pm$ 2.9	31.0 $\pm$ 2.9	20.0 $\pm$ 3.2	9.3 $\pm$ 2.2	7.5 $\pm$ 1.3

Note :

# : Actual initial heavy metal concentration determined by ICP-AES

@ : OD determined by Multiskan MCC/340 MKII

\* : cell count  $\times 10^4$  cells.mL<sup>-1</sup>

a : estimated from OD<sub>620nm</sub> by calculation using regression equation :  $y=3989.4x-22.0$

b : determined by direct cell count of a randomly selected test sample from triplicate, using haemocytometer

Appendix 14 (3a) : Raw data of manganese toxicity tests with *Tetraselmis tetrahele*, conducted in multiwell plates, in the absence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	6.25	12.5	25.0	50.0	100.0
F3.1	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0924	5.9	11.8	23.2	46.0	97.7
	Initial pH	8.0	8.0	8.0	8.0	8.0	8.0
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.062 $\pm$ 0.009	0.048 $\pm$ 0.006	0.042 $\pm$ 0.008	0.026 $\pm$ 0.002	0.015 $\pm$ 0.001	0.017 $\pm$ 0.003
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	36.5 $\pm$ 5.7	27.5 $\pm$ 3.8	23.8 $\pm$ 4.9	13.3 $\pm$ 1.5	5.7 $\pm$ 0.8	7.4 $\pm$ 1.6
F3.2	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	30.8 $\pm$ 2.2	25.2 $\pm$ 2.4	22.0 $\pm$ 2.2	11.3 $\pm$ 1.9	6.0 $\pm$ 1.4	6.8 $\pm$ 1.3
	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0910	5.2	14.9	25.6	49.5	98.1
	Initial pH	8.0	7.8	7.9	8.0	7.9	7.9
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.086 $\pm$ 0.002	0.084 $\pm$ 0.006	0.073 $\pm$ 0.003	0.031 $\pm$ 0.002	0.034 $\pm$ 0.002	0.014 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	51.7 $\pm$ 1.4	50.6 $\pm$ 3.6	43.3 $\pm$ 1.8	16.1 $\pm$ 1.0	18.2 $\pm$ 1.2	5.5 $\pm$ 0.3
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	48.0 $\pm$ 1.6	49.8 $\pm$ 1.7	43.0 $\pm$ 2.4	16.8 $\pm$ 1.9	20.0 $\pm$ 3.3	4.8 $\pm$ 1.5

Appendix 14 (3b) : Raw data of manganese toxicity tests with *Tetraselmis tetrahele*, conducted in flasks, in the absence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	6.25	12.5	25.0	50.0	100.0
F3.3	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0910	5.2	14.9	25.6	49.5	98.1
	Initial pH	8.0	7.8	7.9	8.0	7.9	7.9
	Final pH (n=3)	9.38 $\pm$ 0.02	9.07 $\pm$ 0.07	8.75 $\pm$ 0.03	8.24 $\pm$ 0.04	8.15 $\pm$ 0.02	7.93 $\pm$ 0.01
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.069 $\pm$ 0.006	0.049 $\pm$ 0.008	0.054 $\pm$ 0.003	0.024 $\pm$ 0.001	0.028 $\pm$ 0.001	0.022 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	40.9 $\pm$ 3.9	27.7 $\pm$ 5.3	31.4 $\pm$ 1.6	11.5 $\pm$ 0.3	14.3 $\pm$ 0.7	10.2 $\pm$ 0.8
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	38.0 $\pm$ 2.9	29.8 $\pm$ 2.5	31.3 $\pm$ 4.2	11.3 $\pm$ 1.5	16.3 $\pm$ 1.5	9.3 $\pm$ 2.2

Note :

# : Actual initial heavy metal concentration determined by ICP-AES

@ : OD determined by Multiskan MCC/340 MKII

\* : cell count  $\times 10^4 \text{ cells.mL}^{-1}$

a : estimated from OD<sub>620nm</sub> by calculation using regression equation :  $y=647.4x-3.8$

b : determined by direct cell count of a randomly selected test sample from triplicate, using haemocytometer

**Appendix 14(4)**

**Appendix 14 (4a) :** Raw data of manganese toxicity tests with *Tetraselmis* sp., conducted in multiwell plates, in the absence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	6.25	12.5	25.0	50.0	100.0
F4.1	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0910	5.2	14.9	25.6	49.5	98.1
	Initial pH	8.0	7.8	7.9	8.0	7.9	7.9
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.089 $\pm$ 0.003	0.070 $\pm$ 0.006	0.066 $\pm$ 0.003	0.025 $\pm$ 0.001	0.028 $\pm$ 0.002	0.014 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	58.3 $\pm$ 2.3	44.1 $\pm$ 4.8	41.0 $\pm$ 2.3	9.8 $\pm$ 0.8	12.1 $\pm$ 1.3	1.7 $\pm$ 0.4
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	58.3 $\pm$ 3.3	37.8 $\pm$ 2.2	35.5 $\pm$ 2.1	11.0 $\pm$ 1.8	11.0 $\pm$ 1.4	0.8 $\pm$ 0.5

**Appendix 14 (4b) :** Raw data of manganese toxicity tests with *Tetraselmis* sp., conducted in flasks, in the absence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	6.25	12.5	25.0	50.0	100.0
F4.2	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	0.0910	5.2	14.9	25.6	49.5	98.1
	Initial pH	8.0	7.8	7.9	8.0	7.9	7.9
	Final pH (n=3)	9.22 $\pm$ 0.08	8.95 $\pm$ 0.05	8.69 $\pm$ 0.05	8.38 $\pm$ 0.01	8.23 $\pm$ 0.02	7.93 $\pm$ 0.01
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.049 $\pm$ 0.001	0.051 $\pm$ 0.004	0.052 $\pm$ 0.003	0.027 $\pm$ 0.001	0.027 $\pm$ 0.001	0.020 $\pm$ 0.002
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	28.1 $\pm$ 0.8	29.4 $\pm$ 2.9	30.6 $\pm$ 2.4	11.3 $\pm$ 0.8	11.3 $\pm$ 0.8	6.0 $\pm$ 1.3
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	29.5 $\pm$ 1.7	26.0 $\pm$ 1.6	32.0 $\pm$ 1.8	10.5 $\pm$ 1.0	13.8 $\pm$ 1.3	5.0 $\pm$ 1.2

Note :

# : Actual initial heavy metal concentration determined by ICP-AES

@ : OD determined by Multiskan MCC/340 MKII

\* : cell count X  $10^4$  cells.mL<sup>-1</sup>

a : estimated from OD<sub>620nm</sub> by calculation using regression equation :  $y=761x-9.2$

b : determined by direct cell count of a randomly selected test sample from triplicate, using haemocytometer

## Appendix 15(1)

**Appendix 15 (1a) :** Raw data of arsenic toxicity tests with *Chaetoceros calcitrans*, conducted in multiwell plates, in the presence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	100	200	400	800	1600
G1.1	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	NA	NA	NA	NA	NA	NA
	Initial pH	8.0	8.0	8.0	8.0	8.1	8.1
	96h OD $\pm$ SD (n=3) @	0.072 $\pm$ 0.004	0.054 $\pm$ 0.003	0.046 $\pm$ 0.002	0.028 $\pm$ 0.003	0.017 $\pm$ 0.001	0.006 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	114.5 $\pm$ 6.3	87.4 $\pm$ 4.4	72.9 $\pm$ 3.5	41.7 $\pm$ 4.6	22.5 $\pm$ 1.7	4.1 $\pm$ 1.0
G1.2	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	106.8 $\pm$ 4.3	85.5 $\pm$ 2.6	66.3 $\pm$ 2.1	38.8 $\pm$ 3.0	19.3 $\pm$ 2.6	3.0 $\pm$ 1.2
	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	NA	NA	NA	NA	NA	NA
	Initial pH	8.0	7.8	7.9	8.0	8.0	8.1
	96h OD $\pm$ SD (n=3) @	0.184 $\pm$ 0.002	0.148 $\pm$ 0.004	0.121 $\pm$ 0.009	0.079 $\pm$ 0.005	0.033 $\pm$ 0.002	0.012 $\pm$ 0.001
G1.3	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	312.9 $\pm$ 4.0	250.5 $\pm$ 6.1	203.0 $\pm$ 15.4	130.2 $\pm$ 9.2	49.8 $\pm$ 2.7	13.3 $\pm$ 1.0
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	308.0 $\pm$ 4.1	246.0 $\pm$ 8.4	196.0 $\pm$ 8.7	124.0 $\pm$ 4.1	54.0 $\pm$ 3.9	12.0 $\pm$ 2.9
Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	25	50	100	200	400
Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	NA	NA	NA	NA	NA	NA	
G1.4	Initial pH	7.9	7.9	8.0	7.9	7.9	8.0
	96h OD $\pm$ SD (n=3) @	0.109 $\pm$ 0.017	0.186 $\pm$ 0.005	0.171 $\pm$ 0.007	0.159 $\pm$ 0.006	0.101 $\pm$ 0.006	0.044 $\pm$ 0.005
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	181.6 $\pm$ 29.7	312.0 $\pm$ 8.2	290.4 $\pm$ 11.3	269.6 $\pm$ 9.6	167.7 $\pm$ 9.6	70.4 $\pm$ 9.5
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	183.0 $\pm$ 5.0	305.5 $\pm$ 4.9	282.3 $\pm$ 6.9	260.3 $\pm$ 6.8	159.0 $\pm$ 3.7	83.8 $\pm$ 1.7
G1.5	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	NA	NA	NA	NA	NA	NA
	Initial pH	7.9	7.9	7.9	7.9	7.9	7.9
	96h OD $\pm$ SD (n=3) @	0.104 $\pm$ 0.020	0.159 $\pm$ 0.002	0.154 $\pm$ 0.002	0.144 $\pm$ 0.004	0.106 $\pm$ 0.002	0.052 $\pm$ 0.002
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	173.0 $\pm$ 33.9	269.0 $\pm$ 3.0	260.9 $\pm$ 3.6	243.5 $\pm$ 6.1	177.5 $\pm$ 3.6	83.3 $\pm$ 7.6
G1.6	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	168.8 $\pm$ 5.7	274.0 $\pm$ 5.9	266.8 $\pm$ 4.3	244.3 $\pm$ 5.0	177.3 $\pm$ 2.2	83.4 $\pm$ 3.6
	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	NA	NA	NA	NA	NA	NA
	Initial pH	7.8	7.8	7.9	7.9	7.9	7.9
	96h OD $\pm$ SD (n=3) @	0.057 $\pm$ 0.001	0.101 $\pm$ 0.004	0.086 $\pm$ 0.004	0.078 $\pm$ 0.007	0.059 $\pm$ 0.005	0.028 $\pm$ 0.001
G1.6	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	92.6 $\pm$ 1.0	168.3 $\pm$ 7.0	143.5 $\pm$ 8.2	127.9 $\pm$ 11.8	96.0 $\pm$ 8.2	42.3 $\pm$ 1.6
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	92.8 $\pm$ 3.3	167.3 $\pm$ 4.6	136.3 $\pm$ 5.1	117.3 $\pm$ 3.1	83.8 $\pm$ 2.8	42.3 $\pm$ 3.0

**Appendix 15 (1b) :** Raw data of arsenic toxicity tests with *Chaetoceros calcitrans*, conducted in flasks, in the presence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	25	50	100	200	400
G1.6	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	NA	NA	NA	NA	NA	NA
	Initial pH	7.8	7.8	7.8	7.9	7.9	7.9
	Final pH (n=3)	9.61 $\pm$ 0.05	9.51 $\pm$ 0.01	9.40 $\pm$ 0.01	9.25 $\pm$ 0.01	9.07 $\pm$ 0.03	8.47 $\pm$ 0.03
	96h OD $\pm$ SD (n=3) @	0.079 $\pm$ 0.002	0.091 $\pm$ 0.002	0.081 $\pm$ 0.002	0.080 $\pm$ 0.001	0.066 $\pm$ 0.004	0.027 $\pm$ 0.002
G1.6	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	130.2 $\pm$ 3.5	151.0 $\pm$ 3.0	133.1 $\pm$ 3.6	131.9 $\pm$ 1.7	107.6 $\pm$ 6.3	39.4 $\pm$ 3.6
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	124.5 $\pm$ 6.5	156.0 $\pm$ 4.7	135.5 $\pm$ 4.7	131.5 $\pm$ 5.5	97.3 $\pm$ 4.1	44.0 $\pm$ 3.6

Note :

# : Actual initial heavy metal concentration determined by ICP-AES (NA=not available)

@ : OD determined by Multiskan MCC/340 MKII

\* : cell count  $\times 10^4 \text{ cells.ml}^{-1}$

a : estimated from  $OD_{\text{test}}$  by calculation using regression equation :  $y=1735x-6.9$

b : determined by direct cell count of a randomly selected test sample from triplicate, using haemocytometer

Appendix 15 (2) : Raw data of arsenic toxicity tests with *Isochrysis galbana*, conducted in multiwell plates, in the presence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	100	200	400	800	1600
G2.1	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	NA	NA	NA	NA	NA	NA
	Initial pH	8.0	8.0	8.0	8.0	8.1	8.1
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.024 $\pm$ 0.001	0.021 $\pm$ 0.001	0.017 $\pm$ 0.001	0.012 $\pm$ 0.001	0.008 $\pm$ 0.001	0.006 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	72.4 $\pm$ 2.3	60.5 $\pm$ 2.3	47.1 $\pm$ 4.6	27.2 $\pm$ 2.3	8.6 $\pm$ 2.3	3.2 $\pm$ 2.3
Exp. No.	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	73.0 $\pm$ 2.9	60.8 $\pm$ 4.3	37.3 $\pm$ 1.0	31.5 $\pm$ 2.4	7.8 $\pm$ 1.7	1.5 $\pm$ 0.5
	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	25	50	100	200	400
G2.2	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	NA	NA	NA	NA	NA	NA
	Initial pH	7.9	7.9	8.0	7.9	7.9	8.0
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.047 $\pm$ 0.002	0.051 $\pm$ 0.003	0.054 $\pm$ 0.001	0.051 $\pm$ 0.002	0.040 $\pm$ 0.002	0.020 $\pm$ 0.002
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	166.8 $\pm$ 6.0	180.1 $\pm$ 11.5	194.7 $\pm$ 2.3	180.1 $\pm$ 10.0	137.6 $\pm$ 6.9	59.1 $\pm$ 6.1
Exp. No.	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	164.8 $\pm$ 5.0	172.3 $\pm$ 4.0	194.0 $\pm$ 5.7	163.8 $\pm$ 7.7	134.0 $\pm$ 6.4	5.7 $\pm$ 6.1
	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	133.5	200.0	300.0	450.0	675.0
G2.3	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	NA	NA	NA	NA	NA	NA
	Initial pH	8.0	8.0	8.0	8.0	8.0	8.0
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.065 $\pm$ 0.003	0.055 $\pm$ 0.002	0.040 $\pm$ 0.003	0.030 $\pm$ 0.002	0.023 $\pm$ 0.003	0.016 $\pm$ 0.002
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	229.3 $\pm$ 13.8	196.1 $\pm$ 8.3	136.3 $\pm$ 12.9	99.0 $\pm$ 8.3	69.8 $\pm$ 12.0	43.1 $\pm$ 6.1
Exp. No.	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	219.0 $\pm$ 5.9	192.3 $\pm$ 7.4	147.0 $\pm$ 2.9	104.0 $\pm$ 5.9	55.3 $\pm$ 4.0	43.0 $\pm$ 2.2

Note :

# : Actual initial heavy metal concentration determined by ICP-AES (NA=not available)

@ : OD determined by Multiskan MCC/340 MKII

\* : cell count  $\times 10^6$  cells. $\text{mL}^{-1}$

a : estimated from OD<sub>max</sub> by calculation using regression equation :  $y=3989.4x-22.0$

b : determined by direct cell count of a randomly selected test sample from triplicate, using haemocytometer

Appendix 15 (3a) : Raw data of arsenic toxicity tests with *Tetraselmis tetrahele*, conducted in multiwell plates, in the presence of EDTA

Exp. No.	Nominal Initial Test Concentration (mgL <sup>-1</sup> )	0	6.25	12.5	25.0	50.0	100.0
G3.1	Actual Initial Test Conc. (mgL <sup>-1</sup> ) <sup>#</sup>	NA	NA	NA	NA	NA	NA
	Initial pH	8.0	7.9	7.9	7.9	7.9	7.8
	96h OD ± SD (n=3) <sup>@</sup>	0.129±0.009	0.106±0.006	0.112±0.006	0.096±0.004	0.079±0.004	0.059±0.002
	Estimated <sup>a</sup> Cell Count ± SD* (n=3)	79.9±5.6	65.0±3.6	68.5±4.1	58.6±2.4	47.3±2.8	34.4±1.3
G3.2	Actual <sup>b</sup> Cell Count ± SD* (n=4)	85.0±3.7	59.5±1.9	72.8±2.2	55.5±1.3	44.8±2.2	34.8±2.1
	Actual Initial Test Conc. (mgL <sup>-1</sup> ) <sup>#</sup>	NA	NA	NA	NA	NA	NA
	Initial pH	7.9	7.9	7.9	7.9	8.0	7.8
	96h OD ± SD (n=3) <sup>@</sup>	0.184±0.001	0.225±0.008	0.198±0.015	0.183±0.005	0.135±0.010	0.057±0.005
G3.3	Estimated <sup>a</sup> Cell Count ± SD* (n=3)	115.5±0.4	141.9±5.1	124.2±9.8	114.5±3.2	83.4±6.6	32.9±2.9
	Actual <sup>b</sup> Cell Count ± SD* (n=4)	117.5±2.9	137.8±3.9	116.3±2.6	116.0±3.7	76.3±2.6	29.0±1.8
	Actual Initial Test Conc. (mgL <sup>-1</sup> ) <sup>#</sup>	NA	NA	NA	NA	NA	NA
	Initial pH	8.1	8.1	8.0	8.0	8.0	8.0
	96h OD ± SD (n=3) <sup>@</sup>	0.167±0.006	0.124±0.012	0.119±0.005	0.115±0.008	0.089±0.002	0.052±0.001
	Estimated <sup>a</sup> Cell Count ± SD* (n=3)	104.1±3.9	76.5±7.9	73.0±3.3	70.6±5.1	53.8±1.3	30.1±0.8
	Actual <sup>b</sup> Cell Count ± SD* (n=4)	101.0±4.4	72.8±1.7	76.0±2.9	77.3±2.2	49.5±2.9	28.8±3.9

Appendix 15 (3b) : Raw data of arsenic toxicity tests with *Tetraselmis tetrahele*, conducted in flasks, in the presence of EDTA

Exp. No.	Nominal Initial Test Concentration (mgL <sup>-1</sup> )	0	6.25	12.5	25.0	50.0	100.0
G3.4	Actual Initial Test Conc. (mgL <sup>-1</sup> ) <sup>#</sup>	NA	NA	NA	NA	NA	NA
	Initial pH	8.1	8.1	8.0	8.0	8.0	8.0
	Final pH (n=3)	9.53±0.02	9.53±0.01	9.31±0.01	9.26±0.02	9.62±0.01	9.31±0.07
	96h OD ± SD (n=3) <sup>@</sup>	0.095±0.011	0.080±0.006	0.064±0.005	0.048±0.004	0.064±0.007	0.050±0.005
	Estimated <sup>a</sup> Cell Count ± SD* (n=3)	57.7±7.0	50.8±2.4	37.8±3.2	27.5±2.3	37.4±4.2	28.6±3.3
	Actual <sup>b</sup> Cell Count ± SD* (n=4)	61.3±2.8	46.8±2.5	35.5±2.1	25.5±3.0	37.8±5.3	27.3±3.0

Note :

# : Actual initial heavy metal concentration determined by ICP-AES (NA=not available)

@ : OD determined by Multiskan MCC/340 MKII

\* : cell count X 10<sup>4</sup> cells.mL<sup>-1</sup>

a : estimated from OD<sub>420nm</sub> by calculation using regression equation : y=647.4x-3.8

b : determined by direct cell count of a randomly selected test sample from triplicate, using haemocytometer

## Appendix 15(4)

**Appendix 15 (4a) :** Raw data of arsenic toxicity tests with *Tetraselmis* sp., conducted in multiwell plates, in the presence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	6.25	12.5	25.0	50.0	100.0
G4.1	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	NA	NA	NA	NA	NA	NA
	Initial pH	7.9	7.9	7.9	7.9	7.9	7.9
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.150 $\pm$ 0.008	0.174 $\pm$ 0.009	0.191 $\pm$ 0.008	0.185 $\pm$ 0.021	0.123 $\pm$ 0.005	0.086 $\pm$ 0.004
	Estimated <sup>a</sup> Cell Count $\pm$ SD <sup>a</sup> (n=3)	104.9 $\pm$ 6.0	123.0 $\pm$ 6.9	136.5 $\pm$ 6.3	131.6 $\pm$ 15.6	84.7 $\pm$ 3.4	56.5 $\pm$ 3.1
	Actual <sup>b</sup> Cell Count $\pm$ SD <sup>b</sup> (n=4)	105.8 $\pm$ 6.8	128.5 $\pm$ 5.8	145.3 $\pm$ 5.3	136.8 $\pm$ 4.3	87.0 $\pm$ 4.2	55.3 $\pm$ 3.6
G4.2	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	NA	NA	NA	NA	NA	NA
	Initial pH	8.1	8.1	8.0	8.0	8.0	8.0
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.121 $\pm$ 0.002	0.113 $\pm$ 0.003	0.111 $\pm$ 0.003	0.091 $\pm$ 0.007	0.098 $\pm$ 0.001	0.042 $\pm$ 0.002
	Estimated <sup>a</sup> Cell Count $\pm$ SD <sup>a</sup> (n=3)	82.9 $\pm$ 1.5	77.1 $\pm$ 1.9	75.3 $\pm$ 2.0	59.8 $\pm$ 5.0	65.1 $\pm$ 0.9	22.7 $\pm$ 1.3
	Actual <sup>b</sup> Cell Count $\pm$ SD <sup>b</sup> (n=4)	81.8 $\pm$ 4.6	74.3 $\pm$ 3.3	73.3 $\pm$ 3.4	62.5 $\pm$ 4.2	62.3 $\pm$ 4.3	23.8 $\pm$ 3.5

**Appendix 15 (4b) :** Raw data of arsenic toxicity tests with *Tetraselmis* sp., conducted in flasks, in the presence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	6.25	12.5	25.0	50.0	100.0
G4.3	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	NA	NA	NA	NA	NA	NA
	Initial pH	8.1	8.1	8.0	8.0	8.0	8.0
	Final pH (n=3)	9.55 $\pm$ 0.04	9.56 $\pm$ 0.02	9.47 $\pm$ 0.04	9.50 $\pm$ 0.06	9.72 $\pm$ 0.05	8.93 $\pm$ 0.04
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.095 $\pm$ 0.007	0.081 $\pm$ 0.004	0.066 $\pm$ 0.006	0.061 $\pm$ 0.005	0.062 $\pm$ 0.001	0.028 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD <sup>a</sup> (n=3)	62.8 $\pm$ 5.3	52.4 $\pm$ 2.7	40.9 $\pm$ 4.6	35.7 $\pm$ 2.7	37.7 $\pm$ 0.9	11.8 $\pm$ 0.5
	Actual <sup>b</sup> Cell Count $\pm$ SD <sup>b</sup> (n=4)	61.5 $\pm$ 5.5	49.5 $\pm$ 2.6	41.3 $\pm$ 4.3	34.5 $\pm$ 4.1	36.8 $\pm$ 2.8	10.5 $\pm$ 2.1

Note :

# : Actual initial heavy metal concentration determined by ICP-AES (NA=not available)

@ : OD determined by Multiskan MCC/340 MKII

\* : cell count X  $10^6$  cells. $\text{mL}^{-1}$

a : estimated from OD<sub>660nm</sub> by calculation using regression equation :  $y = 761x - 9.2$

b : determined by direct cell count of a randomly selected test sample from triplicate, using haemocytometer

Appendix 16 (1a) : Raw data of arsenic toxicity tests with *Chaetoceros calcitrans*, conducted in multiwell plates, in the absence of EDTA

Exp. No.	Nominal Initial Test Concentration (mgL <sup>-1</sup> )	0	100	200	400	800	1600
H1.1	Actual Initial Test Conc. (mgL <sup>-1</sup> ) <sup>#</sup>	NA	NA	NA	NA	NA	NA
	Initial pH	7.9	8.0	8.0	8.0	8.1	8.1
	96h OD ± SD (n=3) <sup>@</sup>	0.059±0.005	0.039±0.003	0.037±0.003	0.022±0.004	0.016±0.003	0.009±0.001
	Estimated <sup>a</sup> Cell Count ± SD* (n=3)	94.9±8.2	60.8±4.6	57.3±5.2	30.7±7.0	21.1±5.5	8.7±1.8
H1.2	Actual <sup>b</sup> Cell Count ± SD* (n=4)	96.5±1.9	63.4±1.5	53.5±2.4	23.5±1.3	20.8±2.5	4.8±1.0
	Actual Initial Test Conc. (mgL <sup>-1</sup> ) <sup>#</sup>	NA	NA	NA	NA	NA	NA
	Initial pH	7.9	7.9	7.9	7.9	7.9	7.9
	96h OD ± SD (n=3) <sup>@</sup>	0.106±0.002	0.125±0.006	0.110±0.001	0.086±0.003	0.051±0.007	0.049±0.001
	Estimated <sup>a</sup> Cell Count ± SD* (n=3)	177.6±3.6	210.0±10.8	184.6±1.0	142.8±4.6	90.8±12.5	78.1±1.8
	Actual <sup>b</sup> Cell Count ± SD* (n=4)	166.3±3.5	193.8±2.9	175.3±3.4	143.8±8.1	98.0±4.8	72.8±2.2

Appendix 16 (1b) : Raw data of arsenic toxicity tests with *Chaetoceros calcitrans*, conducted in flasks, in the absence of EDTA

Exp. No.	Nominal Initial Test Concentration (mgL <sup>-1</sup> )	0	100	200	400	800	1600
H1.3	Actual Initial Test Conc. (mgL <sup>-1</sup> ) <sup>#</sup>	NA	NA	NA	NA	NA	NA
	Initial pH	7.9	7.9	8.2	8.2	8.1	8.2
	Final pH (n=3)	9.34±0.68	9.32±0.05	9.21±0.01	8.34±0.04	8.09±0.01	7.74±0.07
	96h OD ± SD (n=3) <sup>@</sup>	0.059±0.002	0.053±0.002	0.050±0.001	0.013±0.001	0.008±0.001	0.006±0.001
	Estimated <sup>a</sup> Cell Count ± SD* (n=3)	94.9±4.0	85.6±2.6	80.5±1.0	16.3±1.0	6.4±1.0	4.1±1.0
	Actual <sup>b</sup> Cell Count ± SD* (n=4)	94.8±3.9	87.8±3.9	81.0±3.9	15.5±3.0	5.5±1.3	1.9±0.1

Note :

# : Actual initial heavy metal concentration determined by ICP-AES (NA=not available)

@ : OD determined by Multiskan MCC/340 MKII

\* : cell count X 10<sup>6</sup> cells.mL<sup>-1</sup>a : estimated from OD<sub>400nm</sub> by calculation using regression equation : y=1735x-6.9

b : determined by direct cell count of a randomly selected test sample from triplicate, using haemocytometer

Appendix 16 (2a) : Raw data of arsenic toxicity tests with *Isochrysis galbana*, conducted in multiwell plates, in the absence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	100	200	400	800	1600
H2.1	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	NA	NA	NA	NA	NA	NA
	Initial pH	7.9	7.9	7.9	8.0	8.0	8.1
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.063 $\pm$ 0.005	0.053 $\pm$ 0.002	0.047 $\pm$ 0.003	0.025 $\pm$ 0.003	0.013 $\pm$ 0.002	0.008 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	229.3 $\pm$ 18.3	189.4 $\pm$ 8.0	164.2 $\pm$ 12.9	76.4 $\pm$ 10.0	31.2 $\pm$ 6.1	8.6 $\pm$ 2.3
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	227.0 $\pm$ 6.3	192.8 $\pm$ 6.8	173.3 $\pm$ 3.9	84.0 $\pm$ 4.5	35.3 $\pm$ 3.8	10.0 $\pm$ 2.2
Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	100.0	150.0	225.0	337.5	506.3
H2.2	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	NA	NA	NA	NA	NA	NA
	Initial pH	7.9	7.9	7.9	7.9	8.0	7.9
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.021 $\pm$ 0.003	0.029 $\pm$ 0.001	0.025 $\pm$ 0.020	0.020 $\pm$ 0.001	0.015 $\pm$ 0.001	0.011 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	63.1 $\pm$ 10.0	93.7 $\pm$ 4.0	79.0 $\pm$ 6.1	57.8 $\pm$ 4.0	89.1 $\pm$ 2.3	21.9 $\pm$ 4.0
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	51.3 $\pm$ 3.0	105.0 $\pm$ 3.6	71.3 $\pm$ 2.2	56.0 $\pm$ 1.8	33.0 $\pm$ 2.2	22.0 $\pm$ 1.8
Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	133.5	200.0	300.0	450.0	675.0
H2.3	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	NA	NA	NA	NA	NA	NA
	Initial pH	7.9	7.9	7.9	7.9	7.9	8.0
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.044 $\pm$ 0.005	0.041 $\pm$ 0.006	0.036 $\pm$ 0.003	0.024 $\pm$ 0.003	0.014 $\pm$ 0.002	0.011 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	154.9 $\pm$ 19.7	140.2 $\pm$ 22.0	121.6 $\pm$ 13.9	73.8 $\pm$ 10.5	35.2 $\pm$ 9.2	21.9 $\pm$ 4.0
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	134.0 $\pm$ 3.9	126.8 $\pm$ 5.6	110.8 $\pm$ 6.5	63.5 $\pm$ 4.8	31.3 $\pm$ 4.3	18.8 $\pm$ 2.5

Appendix 16 (2b) : Raw data of arsenic toxicity tests with *Isochrysis galbana*, conducted in flasks, in the absence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	100	200	400	800	1600
H2.4	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	NA	NA	NA	NA	NA	NA
	Initial pH	8.0	8.0	8.0	8.0	8.0	7.9
	Final pH (n=3)	8.75 $\pm$ 0.08	8.42 $\pm$ 0.01	8.60 $\pm$ 0.05	8.26 $\pm$ 0.02	8.07 $\pm$ 0.03	7.66 $\pm$ 0.21
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.040 $\pm$ 0.002	0.025 $\pm$ 0.001	0.037 $\pm$ 0.002	0.019 $\pm$ 0.001	0.009 $\pm$ 0.001	0.006 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	136.3 $\pm$ 8.3	77.7 $\pm$ 4.0	124.3 $\pm$ 8.3	52.5 $\pm$ 4.6	12.5 $\pm$ 4.5	3.2 $\pm$ 2.3
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	129.0 $\pm$ 4.9	77.3 $\pm$ 4.6	115.5 $\pm$ 2.6	47.8 $\pm$ 3.0	7.8 $\pm$ 1.0	1.5 $\pm$ 0.6

Note :

# : Actual initial heavy metal concentration determined by ICP-AES (NA=not available)

@ : OD determined by Multiskan MCC/340 MKII

\* : cell count  $\times 10^4 \text{ cells.ml}^{-1}$ a : estimated from OD<sub>620nm</sub> by calculation using regression equation :  $y=3989.4x-22.0$ 

b : determined by direct cell count of a randomly selected test sample from triplicate, using haemocytometer

Appendix 16 (3a) : Raw data of arsenic toxicity tests with *Tetraselmis tetrahele*, conducted in multiwell plates, in the absence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	6.25	12.5	25.0	50.0	100.0
H3.1	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	NA	NA	NA	NA	NA	NA
	Initial pH	7.9	7.9	7.9	7.9	7.9	8.0
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.048 $\pm$ 0.005	0.035 $\pm$ 0.001	0.033 $\pm$ 0.001	0.033 $\pm$ 0.003	0.026 $\pm$ 0.003	0.022 $\pm$ 0.004
	Estimated <sup>*</sup> Cell Count $\pm$ SD* (n=3)	27.3 $\pm$ 3.3	19.1 $\pm$ 0.8	17.4 $\pm$ 0.4	15.8 $\pm$ 1.4	13.3 $\pm$ 2.0	10.2 $\pm$ 2.7
H3.2	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	27.0 $\pm$ 2.6	19.0 $\pm$ 0.8	15.0 $\pm$ 0.8	16.0 $\pm$ 1.8	10.5 $\pm$ 1.3	10.3 $\pm$ 1.7
	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	NA	NA	NA	NA	NA	NA
	Initial pH	7.9	8.0	7.9	7.9	7.9	7.9
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.143 $\pm$ 0.005	0.111 $\pm$ 0.010	0.100 $\pm$ 0.006	0.082 $\pm$ 0.005	0.077 $\pm$ 0.007	0.048 $\pm$ 0.003
H3.3	Estimated <sup>*</sup> Cell Count $\pm$ SD* (n=3)	89.0 $\pm$ 3.1	68.3 $\pm$ 6.4	61.1 $\pm$ 3.9	49.3 $\pm$ 3.0	43.2 $\pm$ 3.5	27.5 $\pm$ 2.0
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	89.5 $\pm$ 3.4	69.8 $\pm$ 3.1	65.8 $\pm$ 2.9	48.0 $\pm$ 2.4	46.0 $\pm$ 2.2	24.3 $\pm$ 1.3
	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	NA	NA	NA	NA	NA	NA
	Initial pH	8.2	8.2	8.2	8.2	8.2	8.2
H3.4	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.174 $\pm$ 0.009	0.135 $\pm$ 0.010	0.108 $\pm$ 0.006	0.093 $\pm$ 0.005	0.065 $\pm$ 0.007	0.042 $\pm$ 0.001
	Estimated <sup>*</sup> Cell Count $\pm$ SD* (n=3)	108.8 $\pm$ 5.5	83.6 $\pm$ 6.2	66.1 $\pm$ 3.9	56.4 $\pm$ 3.2	41.2 $\pm$ 6.2	23.1 $\pm$ 0.8
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	100.3 $\pm$ 3.3	79.8 $\pm$ 3.3	70.0 $\pm$ 1.8	53.0 $\pm$ 1.8	33.5 $\pm$ 2.1	22.8 $\pm$ 2.1
	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	NA	NA	NA	NA	NA	NA
H3.5	Initial pH	8.0	8.0	7.9	7.9	7.9	7.9
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.096 $\pm$ 0.008	0.066 $\pm$ 0.003	0.063 $\pm$ 0.008	0.053 $\pm$ 0.005	0.041 $\pm$ 0.006	0.042 $\pm$ 0.003
	Estimated <sup>*</sup> Cell Count $\pm$ SD* (n=3)	58.6 $\pm$ 4.9	38.7 $\pm$ 2.1	37.0 $\pm$ 5.1	30.5 $\pm$ 3.3	22.5 $\pm$ 4.5	23.4 $\pm$ 1.7
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	59.3 $\pm$ 2.5	39.3 $\pm$ 2.8	41.0 $\pm$ 3.7	27.3 $\pm$ 1.9	20.0 $\pm$ 2.9	24.3 $\pm$ 1.5

Appendix 16 (3b) : Raw data of arsenic toxicity tests with *Tetraselmis tetrahele*, conducted in flasks, in the absence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	6.25	12.5	25.0	50.0	100.0
H3.6	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	NA	NA	NA	NA	NA	NA
	Initial pH	8.0	8.0	8.0	8.0	8.0	8.0
	Final pH (n=3)	9.77 $\pm$ 0.04	9.76 $\pm$ 0.03	9.14 $\pm$ 0.02	9.06 $\pm$ 0.06	8.84 $\pm$ 0.02	8.80 $\pm$ 0.06
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.086 $\pm$ 0.001	0.097 $\pm$ 0.005	0.053 $\pm$ 0.001	0.052 $\pm$ 0.002	0.041 $\pm$ 0.006	0.042 $\pm$ 0.001
	Estimated <sup>*</sup> Cell Count $\pm$ SD* (n=3)	51.9 $\pm$ 0.7	59.0 $\pm$ 3.2	30.3 $\pm$ 0.3	30.1 $\pm$ 1.0	26.2 $\pm$ 3.8	23.4 $\pm$ 0.7
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	51.0 $\pm$ 4.8	60.3 $\pm$ 2.6	30.8 $\pm$ 4.6	27.3 $\pm$ 2.8	29.0 $\pm$ 2.9	23.0 $\pm$ 1.8

Note :

# : Actual initial heavy metal concentration determined by ICP-AES (NA=not available)

@ : OD determined by Multiskan MCC/340 MKII

\* : cell count  $\times 10^4 \text{ cells.ml}^{-1}$

a : estimated from OD<sub>96h</sub> by calculation using regression equation :  $y=647.4x-3.8$

b : determined by direct cell count of a randomly selected test sample from triplicate, using haemocytometer

## Appendix 16(4)

**Appendix 16 (4a) :** Raw data of arsenic toxicity tests with *Tetraselmis* sp., conducted in multiwell plates, in the absence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	6.25	12.5	25.0	50.0	100.0
H4.1	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	NA	NA	NA	NA	NA	NA
	Initial pH	7.9	7.9	7.8	7.9	7.8	7.8
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.085 $\pm$ 0.017	0.074 $\pm$ 0.011	0.064 $\pm$ 0.010	0.050 $\pm$ 0.004	0.022 $\pm$ 0.001	0.014 $\pm$ 0.001
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	55.2 $\pm$ 13.1	47.4 $\pm$ 8.6	39.5 $\pm$ 7.7	28.6 $\pm$ 2.9	7.8 $\pm$ 0.5	1.2 $\pm$ 0.5
H4.2	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	53.5 $\pm$ 4.2	42.0 $\pm$ 3.2	33.5 $\pm$ 1.9	28.8 $\pm$ 3.0	7.8 $\pm$ 1.7	2.3 $\pm$ 0.5
	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	NA	NA	NA	NA	NA	NA
	Initial pH	7.9	7.9	7.9	7.9	7.9	8.0
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.045 $\pm$ 0.003	0.030 $\pm$ 0.003	0.030 $\pm$ 0.004	0.032 $\pm$ 0.004	0.023 $\pm$ 0.001	0.014 $\pm$ 0.001
H4.3	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	25.3 $\pm$ 2.3	13.9 $\pm$ 2.3	13.4 $\pm$ 2.7	15.2 $\pm$ 1.6	8.6 $\pm$ 0.5	1.7 $\pm$ 0.4
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	25.0 $\pm$ 1.8	15.5 $\pm$ 1.3	10.0 $\pm$ 1.8	13.4 $\pm$ 1.5	8.5 $\pm$ 1.3	1.0 $\pm$ 0.8
	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	NA	NA	NA	NA	NA	NA
	Initial pH	7.9	8.0	7.9	7.9	7.9	7.9
H4.4	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.117 $\pm$ 0.010	0.102 $\pm$ 0.009	0.089 $\pm$ 0.012	0.082 $\pm$ 0.006	0.064 $\pm$ 0.008	0.030 $\pm$ 0.005
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	79.6 $\pm$ 7.6	68.2 $\pm$ 6.6	58.3 $\pm$ 8.9	53.2 $\pm$ 4.6	39.2 $\pm$ 6.4	13.3 $\pm$ 3.5
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	87.5 $\pm$ 2.5	66.0 $\pm$ 1.8	66.0 $\pm$ 3.2	54.3 $\pm$ 1.7	31.3 $\pm$ 3.0	10.3 $\pm$ 1.5
	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	NA	NA	NA	NA	NA	NA
H4.5	Initial pH	8.0	8.0	8.0	8.0	8.0	8.0
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.103 $\pm$ 0.011	0.113 $\pm$ 0.014	0.085 $\pm$ 0.004	0.071 $\pm$ 0.008	0.039 $\pm$ 0.007	0.019 $\pm$ 0.002
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	69.0 $\pm$ 8.8	76.6 $\pm$ 10.8	55.5 $\pm$ 2.8	44.6 $\pm$ 5.8	20.2 $\pm$ 5.6	5.5 $\pm$ 1.7
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	60.5 $\pm$ 2.5	69.0 $\pm$ 3.2	52.0 $\pm$ 1.8	40.3 $\pm$ 2.4	19.3 $\pm$ 2.5	4.0 $\pm$ 0.8

**Appendix 16 (4b) :** Raw data of arsenic toxicity tests with *Tetraselmis* sp., conducted in flasks, in the absence of EDTA

Exp. No.	Nominal Initial Test Concentration ( $\text{mgL}^{-1}$ )	0	6.25	12.5	25.0	50.0	100.0
H4.5	Actual Initial Test Conc. ( $\text{mgL}^{-1}$ ) <sup>#</sup>	NA	NA	NA	NA	NA	NA
	Initial pH	8.0	8.0	8.0	8.0	8.0	8.0
	Final pH (n=3)	9.75 $\pm$ 0.02	9.72 $\pm$ 0.05	9.41 $\pm$ 0.02	0.22 $\pm$ 0.09	9.16 $\pm$ 0.08	8.73 $\pm$ 0.14
	96h OD $\pm$ SD (n=3) <sup>@</sup>	0.084 $\pm$ 0.001	0.098 $\pm$ 0.004	0.053 $\pm$ 0.006	0.055 $\pm$ 0.006	0.041 $\pm$ 0.002	0.040 $\pm$ 0.003
	Estimated <sup>a</sup> Cell Count $\pm$ SD* (n=3)	54.5 $\pm$ 0.9	65.6 $\pm$ 2.9	31.1 $\pm$ 4.6	32.4 $\pm$ 4.2	21.8 $\pm$ 1.8	21.5 $\pm$ 2.5
	Actual <sup>b</sup> Cell Count $\pm$ SD* (n=4)	56.0 $\pm$ 4.3	62.3 $\pm$ 2.2	31.5 $\pm$ 3.4	38.5 $\pm$ 5.0	22.3 $\pm$ 3.3	21.8 $\pm$ 2.1

Note :

# : Actual initial heavy metal concentration determined by ICP-AES (NA=not available)

@ : OD determined by Multiskan MCC/340 MKII

\* : cell count  $\times 10^4$  cells.ml $^{-1}$

a : estimated from OD<sub>max</sub> by calculation using regression equation :  $y=761x-9.2$

b : determined by direct cell count of a randomly selected test sample from triplicate, using haemocytometer

## Appendix 17 : Reference toxicant ( Cd ) control chart data

## (1) Cd with EDTA

Test species	Mean IC <sub>50</sub> (mgL <sup>-1</sup> )	95% Confidence limits (Mean±2SD)	99.7% Confidence limits (Mean±3SD)
<i>C. calcitrans</i>	13.9 (4)	7.2 - 20.6	3.9 - 23.9
<i>I. galbana</i>	4.7 (2)	2.7 - 6.8	1.7 - 7.8
<i>T. tetrahele</i>	18.1 (2)	16.6 - 19.7	15.8 - 20.5
T. sp.	15.5 (3)	8.5 - 22.5	4.9 - 26.0

Note : ( ) = number of experiments

## (2) Cd without EDTA

Test species	Mean IC <sub>50</sub> (mgL <sup>-1</sup> )	95% Confidence limits (Mean±2SD)	99.7% Confidence limits (Mean±3SD)
<i>C. calcitrans</i>	0.06 (2)	0.03 - 0.09	0.02 - 0.10
<i>I. galbana</i>	0.06 (3)	0.04 - 0.08	0.03 - 0.09
<i>T. tetrahele</i>	5.7 (3)	3.2 - 8.2	1.9 - 9.5
T. sp.	5.7 (5)	2.5 - 8.8	0.9 - 10.4

Note : ( ) = number of experiments