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Metal to phosphorus stoichiometries for freshwater phytoplankton in three Scottish lakes

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Abstract

Simultaneous measurements of changes in phytoplankton biomass and the metal and phosphorus (P) content of cells have been captured to attest metal to P stoichiometries for freshwater phytoplankton. Three remote Scottish lakes that have received high, medium or low metal contamination from the atmosphere were selected for study. Phytoplankton cells were collected, their biomass determined microscopically, and Inductively Coupled Plasma-Mass Spectrometry was used to measure their lead (Pb), cadmium (Cd), mercury (Hg), copper (Cu), zinc (Zn), nickel (Ni), chromium (Cr), manganese (Mn), cobalt (Co) and P content. A greater phytoplankton biomass in the lakes resulted in significant algae growth dilution of the mass-specific Pb, Cd, Hg, Cu, Ni and Cr in the phytoplankton. Changes in the phytoplankton cell count and their Hg, Pb, Cd, Cu, Mn, Co, Ni and Cr concentrations showed the process of algae bloom dilution to be subject to exponential decay, which accelerated in the order of Mn < Cu < Ni < Pb and Cd < Cr and Hg < Co. This indicated a metabolic and detoxification mechanism was involved in the active selection of metals. For the first time simultaneous measurements of metals and P stoichiometry in freshwater phytoplankton are reported. The mean metal to P stoichiometry generated was $(C_{106}P_1N_{16})_{1000}Pb_{0.019}Hg_{0.00004}Cu_{0.013}Cd_{0.005}Cr_{0.2}Co_{0.0008}\,Mn_{0.2}Ni_{0.012}$ based on field measurements and the Redfield average C, N and P stoichiometry of $(CH_2O)_{106}(NH_3)_{16}H_3PO_4$.

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Introduction

The majority of phytoplankton cells are typically composed of carbon (C), nitrogen (N) and phosphorus (P) and have a commonly accepted average stoichiometry of $(CH_2O)_{106}(NH_3)_{16}H_3PO_4$ (Redfield et al., 1963; Sanudo-Wilhelmy et al., 2004). In the same context, phytoplankton can exploit iron (Fe), manganese (Mn), zinc (Zn), copper (Cu) and nickel (Ni) for N acquisition, oxygen cycling, chlorophyll synthesis, and sulfate reduction (Moffett et al., 1997; Twining et al., 2004). These nutrient metals can be replaced at their metabolic site by toxic metals such as cadmium (Cd), mercury (Hg), lead (Pb) and chromium (Cr) (Bruland et al., 1978; Sunda and Huntsman, 1998).

The cells can accumulate metals because they have a large surface area that has hydrophilic groups or hydroxy complexes with O-containing donor groups (-COH: -COOH; -$P(O)(OH)_2$), which bind to ambient metal cations (Vasconcelos et al., 2002). These sites on the cell surface are ligands from which metals can either dissociate back into solution or travel into the cytoplasm (Sunda and Huntsman, 1998). This has been reported as a dominant process of trace metal removal from solution (Whitfield, 2001; Lohan et al., 2005). Alternatively, cellular metal update may also occur through transport proteins or porins that are embedded in the outer membrane and allow for non-selective passive diffusion of metal ions across the outer membrane (Ma et al., 2009).

Due to the realisation of the proclivity of metals to bind non-specifically to cell surfaces, studies have extended Redfield et al.'s stoichiometric composition of phytoplankton to include metals. Ho et al. (2003) calculated a mean stoichiometry (mol:mol) of $(C_{124}N_{16}P_1S_{1.3}K_{1.7}Mg_{0.56}Ca_{0.5})_{1000}Sr_{5.0}Fe_{7.5}Zn_{0.80}Cu_{0.38}Co_{0.19}Cd_{0.21}Mo_{0.03}$*, while Twining et al. (2004) found $(C_{72}P_1S_{0.70})_{1000}Zn_{5.4}Fe_{1.8}Ni_{0.61}Mn_{0.26}$ for marine phytoplankton. Yet

* Sulphur (S), potassium (K), magnesium (Mg), calcium (Ca), strontium (Sr), cobalt (Co), molybdenum (Mo).

simultaneous measurements of metal to P stoichiometry in freshwater phytoplankton have only been estimated (Wang and Dei, 2006).

Sigg (1985, 1987) presented mean stoichiometries of $C_{113}P_1Zn_{0.06}Cu_{0.008}$ and $(CH_2O)_{106}(NH_3)_{16}H_3PO_4Cu_{0.0006}Zn_{0.03}$ for the phytoplankton of Lake Constance and Lake Zurich (Switzerland) respectively. However, the mean surface areas of the algae cells were estimated from correlation of the organic material content of the settling particles using typical cell dimensions of diatoms. Sigg therefore acknowledged the stoichiometries to be an approximation that would vary if different algal species were taken into account. Likewise, when Reynolds and Hamilton-Taylor (1992) calculated a stoichiometry of $C_{106}P_1Zn_{0.034}$ for Lake Windermere, United Kingdom (UK), they estimated P based on regression data of dissolved P concentrations and the C: Si atomic ratio of 1:0.40 in phytoplankton cells.

Recommendations have been made that metal to P stoichiometries be incorporated into Biotic Ligand Models (BLM) (De Schamphelaere et al., 2005). When BLM were first developed, they provided a way to predict the ambient metal concentration that will have an effect (e.g. lethality) on organisms (e.g. fish), and emphasised the importance of including ligand concentration (e.g. fish gills) for that prediction (Di Toro et al., 2002). The models assumed a fixed rate of metal uptake occurred according to ambient concentrations, thus they were extended to include ambient water chemistry (Paquin et al., 2002). De Schamphelaere et al. (2005) then showed that cellular metal concentrations were better than ambient metal concentrations for predicting the threat of toxicity to freshwater phytoplankton. They stressed that cell surfaces should be used as the ligand for metals in the same way as fish gills apply to the BLM for predicting metal toxicity to fish species. Wang and Dei (2006) then showed that the metal to nutrient stoichiometry in phytoplankton cells better predicts metal toxicity than cellular metal burden. Therefore, the need for a simultaneous measurement of metal to nutrient (in this case P) stoichiometry in freshwater phytoplankton will be addressed here.

Site descriptions

Investigations were undertaken in three lakes that have been shown to receive varying degrees of metal contamination in the UK (Rippey and Douglas, 2004). That is, one lake was selected in a region that receives high atmospheric metal contamination, one lake was selected in a region that receives medium atmospheric metal contamination, and one lake was selected in a region that receives low atmospheric metal contamination. Due to the need for appropriate lacustrine data on the relationship between metals in the phytoplankton and the dissolved phase (Reynolds & Hamilton-Taylor, 1992; Chen & Folt, 2005; Croteau *et al*., 2005; Wang & Dei, 2006), it was considered important to obtain such data from a range of metal contaminated regions in order to address any variations. The three lakes are also in remote catchments with slowly weathering rocks and poorly buffered waters (Flower et al., 1994), and receive metal contamination solely from atmospheric deposition (Rippey and Douglas, 2004). This was the main reason they were selected for investigation because capturing metal-nutrient interactions in lakes that receive metal contamination from runoff or direct discharges would be problematic (Murray, 1987).

In the following site descriptions, lake surface area, perimeter, altitude, grid reference, catchment area, maximum basin relief, and distance from the sea and to the nearest village were calculated and/or obtained using the OS Landranger® Memory-Map™ V5 edition (2006) for northern and southern Scotland (Licence number PU 100034184). The maximum lake depths were based on collected field data, while catchment geology, vegetation and soil type were derived from Patrick *et al.* (1991, 1995).

Loch Coire nan Arr has a surface area of 13.21 ha, a maximum lake depth of 11 m and a catchment area of 8.45 km^2 (Table 1). It is the most northerly of the three sites and lies in the region of low metal contamination from the atmosphere (Rippey and Douglas, 2004). The catchment is dominated by steep corrie cliffs, and the lake itself fills a large deep sandstone

corrie that was carved by deglaciation at the end of the Pleistocene. Loch Coire nan Arr is one of the six UK sites represented in the UNECE International Co-operative programme on Assessment and Monitoring of Acidification of Rivers and Lakes (Juggins *et al*., 1996). Permission for sampling the site was obtained from The Applecross Trust, a conservation charity responsible for the management of the lake (contact: admin@applecross.org.uk).

Loch Doilet has a surface are of 51.55 ha, a maximum lake depth of 16 m and a catchment area of 33.51 km^2 (Table 1). The lake, lying northwest of the Ben Nevis Mountain range, is the largest of the three lakes and has received moderate metal contamination from the atmosphere (Rippey and Douglas, 2004). The catchment rises from the lake to a peak of approximately 720 m. The dominant soil types are peats, which are eroded on the uppermost reaches of the catchment (Patrick et al., 1995). Permission for sampling the site was obtained from the Forestry Commission Scotland, a UK non-ministerial government department responsible for the management of the lake (contact: lochaber@forestry.gsi.gov.uk).

Loch Urr has a surface area of 47 ha with a maximum lake depth of 13 m (Table 1). It lies in the Dumfries and Galloway region of south-west Scotland, an area that has received high metal contamination from the atmosphere (Rippey and Douglas, 2004). The lake drains the smallest of the three catchments with an area of 7.73 km^2. The underlying geology is complicated by is mainly composed of granite / gneiss and the land-use is confined to low-intensity sheep grazing (Patrick *et al*., 1991). Permission for sampling the site was obtained from the Urr District Salmon Fisheries Board, a board of the Galloway Fisheries Trust charity set up to protect the lake and its catchment (contact: mail@gallowayfisheriestrust.org).

Table 1. Summary of the site characteristics of Loch Coire nan Arr in northwestern Scotland, Loch Doilet in western Scotland and Loch Urr in southern Scotland.

	Loch Coire nan Arr	Loch Doilet	Loch Urr
Grid Reference	NG 808422	NM807677	NX759864
Surface area	13.21 ha	51.55 ha	47.0 ha
Perimeter	1.86 km	5.49 km	4.2 km
Maximum lake depth	11 m	16 m	13.2 m
Lake volume	5.6×10^5 m^3	4.1×10^6 m^3	2.35×10^6 m^3
Distance upstream from sea	2.03 km	6.2 km	22.7 km
Aerial distance from nearest village	8.91 km (Lochcarron)	8.84 km (Strontian)	6.6 km (Monaive)
Elevation/altitude	125 m	8 m	193 m
Catchment area	8.45 km^2	33.51 km^2	7.73 km^2
Catchment geology	Torridonian Sandstone	Schists and gneiss	Granite / gneiss
Catchment vegetation	Confiers < 1 %	Conifers – 50 %, moorland – 50 %	Moorland – 100 %
Catchment soils	Peat	Peats	Podsol, peaty gley blanket peat

Sampling

Sampling campaigns were conducted on ten occasions over a 16 month period from June 2006 to September 2007. Before fieldwork, all sample containers were prepared to reduce metal contamination and prevent adsorption losses to the container walls (Yu et al., 2003).

During fieldwork, three lake water samples were collected from each lake. The first sample was for the analysis of chlorophyll-*a*, total phosphorus (TP) and pH. The second was for analysis of total metal concentrations. The third was for phytoplankton identification and calculations of biomass. The water was taken from a central location (6 m) near the deepest point of the lake using a Perspex Ruttner sampler, as recommended by Sykes et al. (1999).

Phytoplankton samples were also collected from the lakes on each of the sampling occasions following the standard principals using the net haul method (Vollenweider, 1974). A 20 μm mesh net (30 cm wide) was used (EB Nets, UK) to take 10 to 18 hauls (varying with lake productivity) of concentrated phytoplankton. An adjustment was made to the standard nets to separate the zooplankton during each haul. Two filters, one of 20 μm and one of 250

142 μm were stacked on top of each other with a 35 mm spacer such that water flowed first
143 through the 250 μm and then the 20 μm filter. The upper filter of mesh 250 μm was a
144 sufficient size to trap the zooplankton but allow the smaller phytoplankton to be trapped in the
145 smaller 20 μm mesh. Separating the plankton in-situ minimised sample handling and
146 therefore the possibility of metal contamination. When the two size fractions were
147 microscopically analysed, the zooplankton were not incorporated into the phytoplankton
148 samples.

149 The water samples collected for phytoplankton identification and biomass calculations
150 were immediately transferred from their LDPE bottles to acid washed scintillation vials (25
151 ml) that were pre-prepared with the fixative glutaraldehyde (Electron Microscopy grade,
152 EMS, Pennsylvania, U.S.A). The glutaraldehyde (50 %) was buffered (pH 8) with 1 M NaOH
153 and diluted with Milli-Q water to 10 % (Twining et al., 2004) before preparing the vials to
154 produce a final concentration of 2 % (v/v) in the sample (Menden-Deuer et al., 2001).

155 The net haul material was transferred to a total of 36 polyethylene acid cleaned
156 sampling vials (32 ml) at each site (AGB Scientific Ltd., UK). The vials used to store the net
157 haul material were also pre-prepared to achieve 2 % glutaraldehyde in the sample, except in
158 this case, the glutaraldehyde was passed through a Dowex 50-W X8-200 cation exchange
159 resin (50X4-400; H-form) to remove trace metals (Twinning et. al., 2004).

160

161 **Sample Analysis**

162 TP concentrations were measured spectrometrically in the digest of the unfiltered
163 sample at 882 nm (Murphy & Riley, 1962; Eisenreich *et al*., 1975). Chlorophyll-*a* was
164 extracted from the filtered samples into 90 % V/V methanol, and the detection was performed
165 with a spectrophotometer set at an emission wavelength of 665 nm (Riemann, 1978). A
166 Shimadzu UV-Mini 1240 Spectrophotometer was used for this at the University of Ulster.

A Nikon-5400 inverted light microscope at 40 x was used to examine the phytoplankton samples and identify the species present. For this, 10 ml of the lake water sample preserved in glutaraldehyde was allowed to sediment in a settling chamber for no less than 8 hours. Blue-green and green algae organisms were identified following the interactive keys produced by Whitton et al. (2002, 2003). For those organisms that proved difficult to distinguish, a more detailed text was consulted, i.e. John et al. (2002). The guidelines presented by Kelly (2000) were followed to identify any cells representative of the Phylum Bacillariophyta and the Phylum Fragilariophyceae (Diatoms).

During identification, the species/genre/groups were also counted and measured for volume and surface area calculations following the procedures described by Olrik et al. (1998). At least 10 length and width measurements were recorded for each species (wall to wall), and when fewer than 10 cells were present, those present were measured. Cell counts were converted to counts per volume of lake water. Cell volumes and surface areas were calculated using the geometric equations of Hillebrand et al. (1999). The volume of colonial and filamentous cells was calculated from the volume of a single cell multiplied by the number of cells in each colony/filament.

The cell surface area and volume calculations were then collated with cell counts per volume of lake water to equate the surface area and biomass per volume of lake water. These calculations were also completed following the guidelines of Olrik et al. (1998). At 40 x magnification, the width of one uninterrupted diagonal across a settling chamber is 2.575 mm, and with a chamber diameter of 23 mm, the area of one counting field is 59.23 mm^2.

To prepare the phytoplankton net haul material for acid digestion, the method followed was that of Reynolds and Hamilton-Taylor (1992). To achieve blank concentrations, 2 x 32 ml vials of 2 % glutaraldehyde were prepared prior to each fieldwork session and brought on fieldwork to ensure they had the same sample exposure. On return to the

laboratory, a stream of Milli-Q water was used to fill the vial as it was passed through the same plankton net filter used to collect the samples.

The phytoplankton samples were made soluble (digested) by treatment with hydrofluoric, nitric and perchloric acid, following the acid digestion technique provided in Bock (1979). An empty beaker (a reagent blank), and two samples of certified reference material (CRM) were included with every batch (between 20-30 samples). The CRM used for this study was Chinese stream sediment (GBW 07301) issued under the laboratory of the Government Chemist (LGC) trademark (LGC Promochem, UK). The digested samples were stored in acid cleaned 25 ml scintillation vials until further analysis with Inductively Coupled Plasma-Mass Spectrometry (ICP-MS).

The XSeries[I] ICP-MS (ThermoFisher Scientific Cooperation) was used for the analysis of metals and P in the samples (Table 1). All prepared standard solutions, samples and blanks were acidified with 2 % (w/v) HNO_3^- (BDH Aristar, AGB Scientific Ltd., UK). The precision of every element was assessed from replicate and, when possible, triplicate analysis of reference material and of samples collected in fieldwork. This was found to be 5% relative standard deviation (RSD) or better, which is generally considered acceptable precision (Long et al., 1990). Also, instrument stability was indicated in the RSD of triplicate ICP-MS measurements for all analytes of less that 5% in all cases, and in many cases less than 2%.

Table 2. Fully quantitative concentrations that showed linearity in the calibration curves computed by Plasmalab. These were subsequently used in the regression analysis to determine the concentration of the elements in the unknown sample solutions.

Std. label	Standard concentrations used for calibration (*μ*g/l)						
	Na	Mg	P	Cr	Mn	Fe	Co
1							0.1

2		1.0		1.0	1.0		1.0
3		10.0		10.0	10.0		10.0
4	100.0	100.0	100.0	100.0	100.0	100.0	100.0
5	1000.0	1000.0	1000.0	1000.0		1000.0	1000.0
6	5000.0						
	Ni	**Cu**	**Zn**	**Cd**	**Hg**	**Pb**	
1	0.1	0.1	0.1	0.1	0.1	0.1	
2	1.0	1.0	1.0	1.0	1.0	1.0	
3	10.0	10.0	10.0	10.0	10.0	10.0	
4	100.0	100.0	100.0	100.0	100.0	100.0	
5	1000.0	1000.0	1000.0	1000.0			
6							

Results

The measured concentrations of chlorophyll-*a* and TP and modelled chlorophyll-*a* concentrations based on OEDC (1982) and Prairie et al. (1989) models for predicting chlorophyll-*a* based on TP concentrations are presented in Figure 1. The peak of TP concentrations was recorded in mid-May 2007 for Loch Doilet (23.5 μg/l) and Coire nan Arr (79.3 μg/l), whereas the peak in Loch Urr (85.3 μg/l) occurred in late September 2006. The chlorophyll-*a* trends in Figure 1 show a peak during August/September 2006 for Loch Doilet (3.10 μg/l) and September 2007 for Loch Urr (23.0 μg/l), whereas the peak in Loch Coire nan Arr was during the month of July 2007 (10.25 μg/l). The lowest chlorophyll-a concentrations were 1.4, 1.5 and 2.7 μg/l respectively for Loch Coire nan Arr, Loch Doilet and Loch Urr. In many cases, Figure 1 shows that an increase in TP is followed by a rise in chlorophyll-a on the subsequent sampling occasion, particularly in Loch Coire nan Arr and Loch Urr. Also, the patterns of chlorophyll-*a* generally show similar timing in their fluctuations to that of the predictions of chlorophyll-*a* concentrations, notably in Loch Doilet.

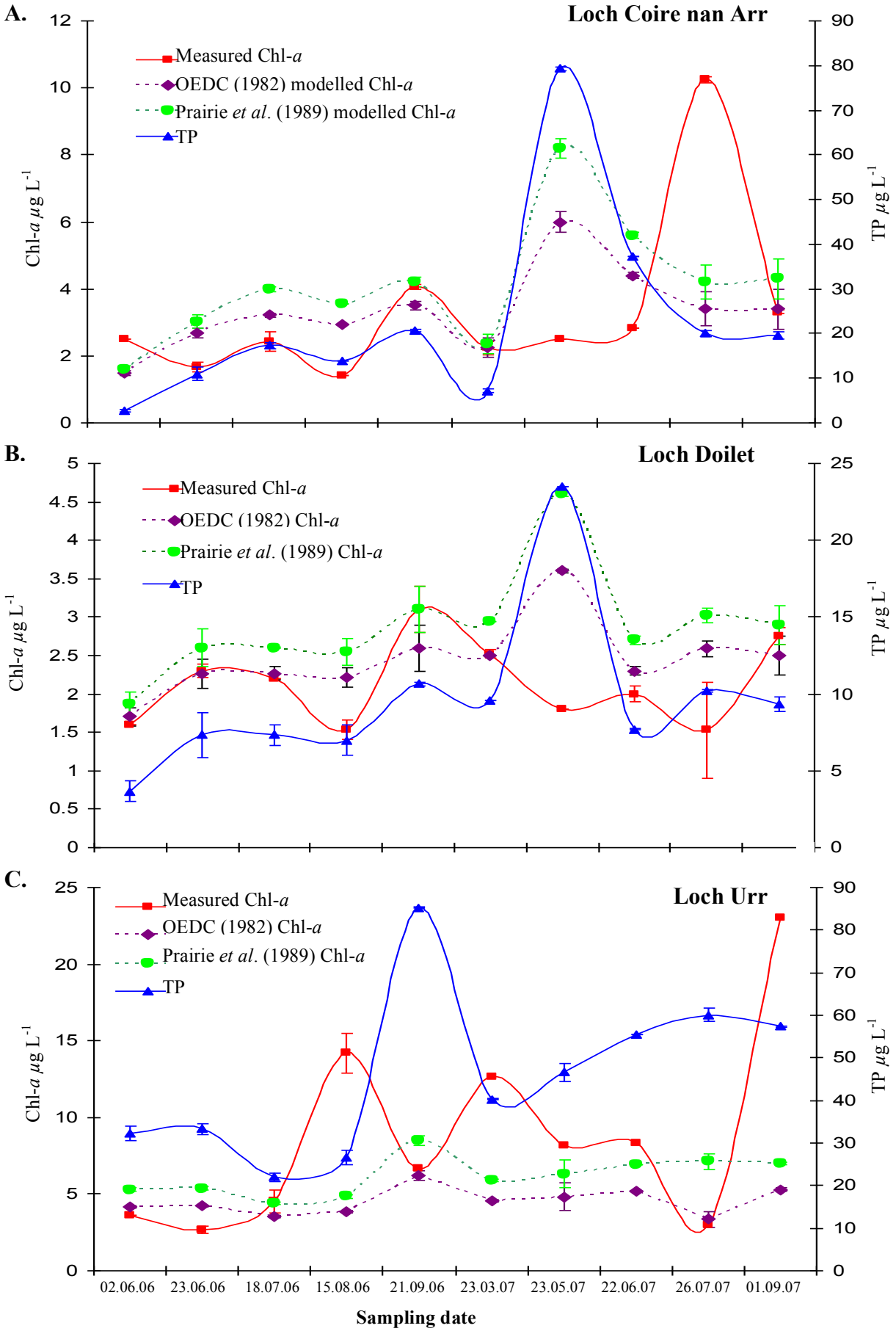
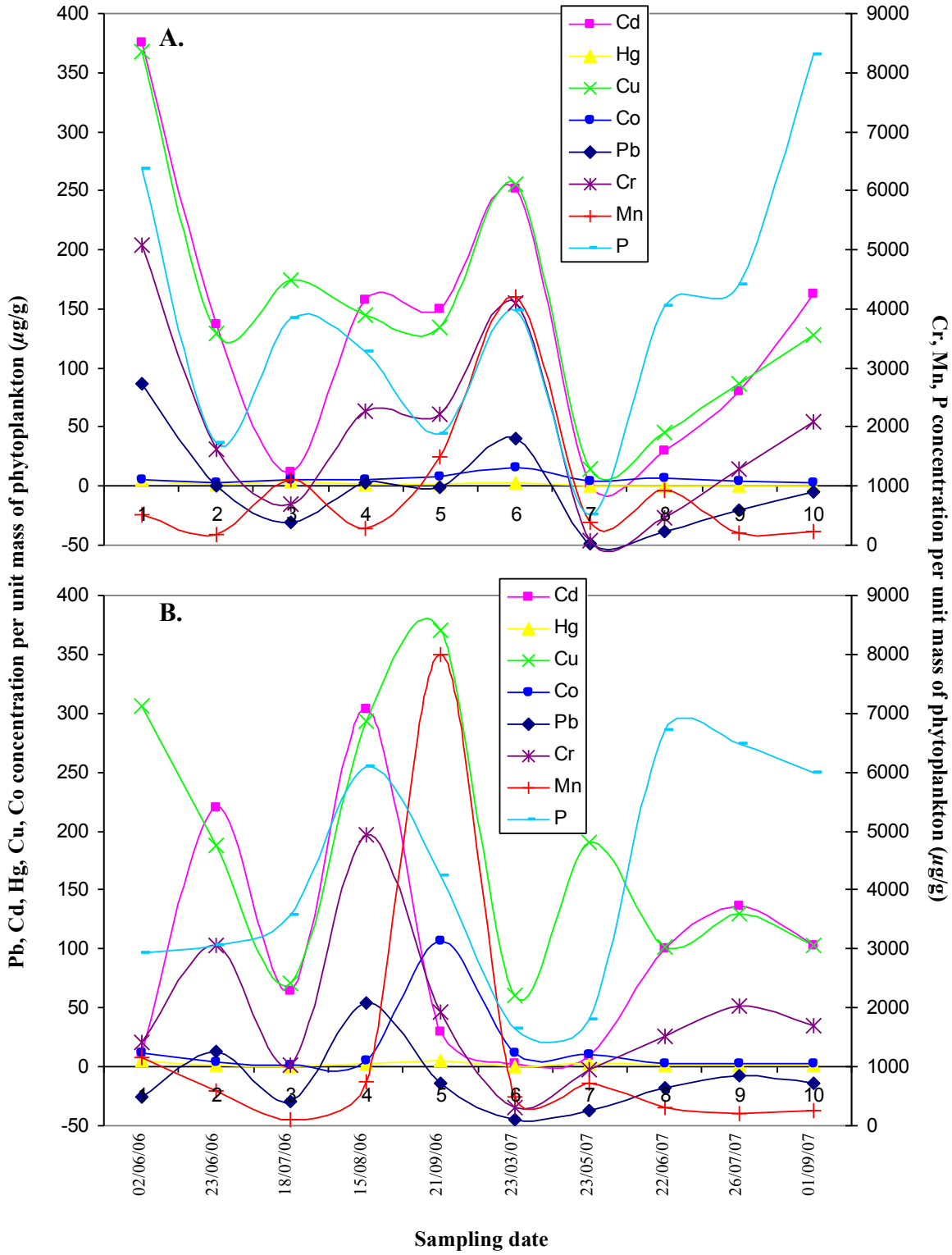


Figure 1. Chlorophyll-*a* (Chl-*a*) and total phosphorus (TP) concentrations measured in Loch Coire nan Arr **(A)**, Loch Doilet **(B)**,and Loch Urr **(C)**. Predicted Chl-*a* concentrations are those estimated with the formulae provided by Prairie *et al*. (1989) and the Organisation for Economic Corporation and Development (OEDC, 1982) using the actual measured TP concentrations. The series keys located in the top left of the diagrams applies to each of the tread lines in Figure 1.A, B and C. Error bars are the standard error between the triplicate measurements of each result (*n*=3).

Figure 2 shows the concentrations of Pb, Hg, Cd, Cu, Cr, Co, Mn and P determined per unit mass of the phytoplankton cells in Loch Coire nan Arr (A), Loch Doilet (B) and Loch Urr (C). The trend lines show high fluctuation across the sampling dates from early June 2006 to September 2007. In Loch Coire nan Arr the maximum concentration of metals recorded in the phytoplankton were 2.73 mg/g (Pb), 0.38 mg/g (Cd), 0.01 mg/g (Hg), 8.30 mg/g (P), 0.37 mg/g (Cu), 5.08 mg/g (Cr), 0.02 mg/g (Co) and 4.20 mg/g (Mn). The minimum concentrations were 38.07 μg/g (Pb), 1.17 μg/g (Cd), 0.18 μg/g (Hg), 510 μg/g (P), 14.8 μg/g (Cu), 79.2 μg/g (Cr), 18.8 μg/g (Co) and 190 μg/g (Mn). For Loch Doilet, the peak concentrations were 2.07 mg/g (Pb), 0.30 mg/g (Cd), 0.01 mg/l (Hg), 6.72 mg/g (P), 3.10 mg/g (Cu), 4.93 mg/g (Cr), 0.10 mg/g (Co) and 8.00 mg/g (Mn). The lowest concentrations were 100 μg/g (Pb), 2.20 μg/g (Cd), 0.65 μg/g (Hg), 1660 μg/g (P), 60.36 μg/g (Cu), 300 μg/g (Cr), 1.32 μg/g (Co), 92.28 μg/g (Mn). In the majority of cases the phytoplankton of Loch Urr held the lowest concentrations of metals, but the highest concentration of P in the cells. The maximum values were 0.33 mg/g (Pb), 0.06 mg/g (Cd), 0.02 mg/g (Hg), 16.21 mg/g (P), 0.22 mg/g (Cu), 0.90 mg/g (Cr), 0.01 mg/g (Co), and 5.75 mg/g (Mn). Minimum concentrations of 85.36 μg/g (Pb), 3.21 μg/g (Cd), 0.25 μg/g (Hg), 1470 μg/g (P), 30.27 μg/g (Cu), 250 μg/g (Cr), 0.60 μg/g (Co), 52.54 μg/g (Mn) were also recorded.


A.
B.
Cd
Hg
Cu
Co
Pb
Cr
Mn
P
Pb, Cd, Hg, Cu, Co concentration per unit mass of phytoplankton (μg/g)
Cr, Mn, P concentration per unit mass of phytoplankton (μg/g)
Sampling date
02/06/06
23/06/06
18/07/06
15/08/06
21/09/06
23/03/07
23/05/07
22/06/07
26/07/07
01/09/07

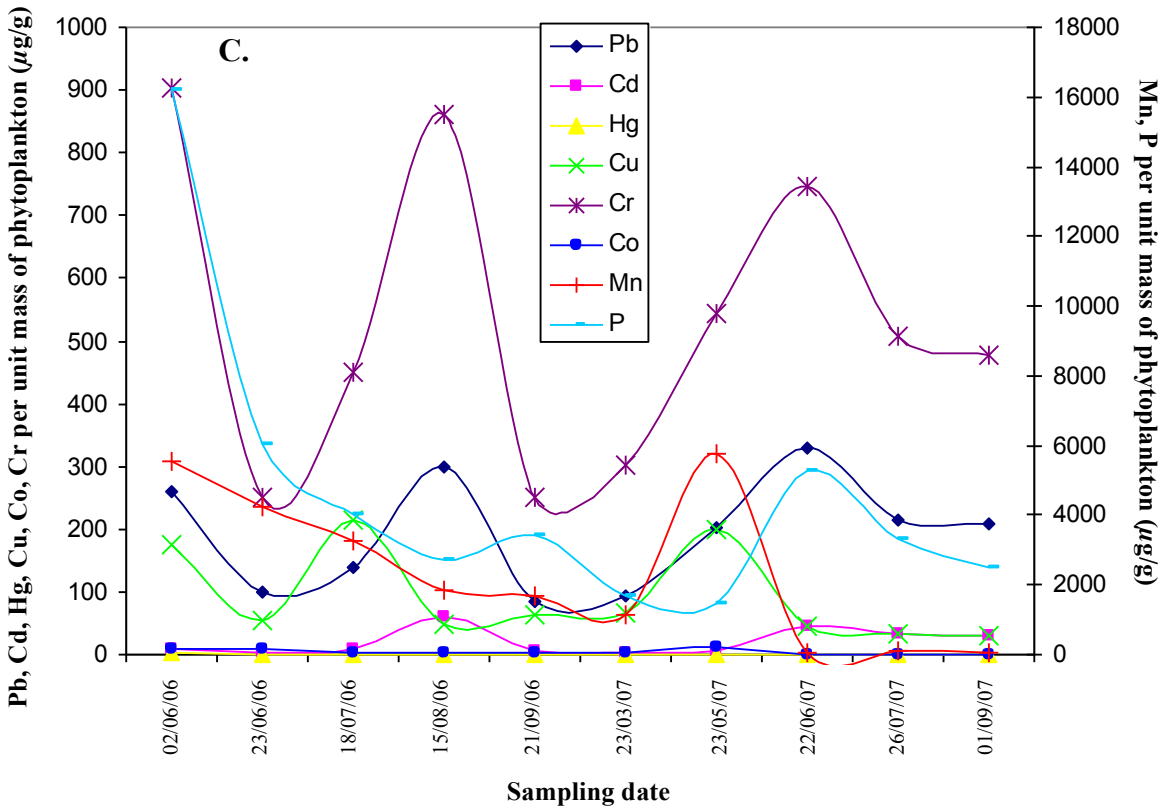


Figure 2. Concentrations of Pb, Hg, Cd, Cu, Cr, Co, Mn and P determined per unit mass of the phytoplankton cells collected in Loch Coire nan Arr **(A)**, Loch Doilet **(B)**,and Loch Urr **(C)**. All values are in μg of metal per g of phytoplankton, with those metals in the higher concentration range detailed on the secondary/right-hand side axis. The series keys located in the central area of the diagrams applies to each of the trend lines in A, B and C.

The concentration of Pb, Cd, Hg, Cr, Cu and Ni per unit mass of phytoplankton cells is plotted against the TP concentrations of the three lakes on all sampling occasions in Figure 3 (n=*29*). The scatterplots show a linear relationship with negative slope between each of the two sets of variables. This indicates that the lower the lake TP concentration, the higher the concentration of metals per unit mass of phytoplankton. Before completing the regression analysis in Figure 3, the Kolmogorov-Smirnov and Shapiro-Wilk's tests on the normality of the (raw) data showed the TP concentrations and the mass-specific metal concentration in the

phytoplankton to not be normally distributed ($p < 0.05$). However, using the log-transformed metal concentrations and the cubic root of TP concentrations, the data showed normal distribution ($p > 0.05$) in the Kolmogorov-Smirnov and Shapiro-Wilk's tests.

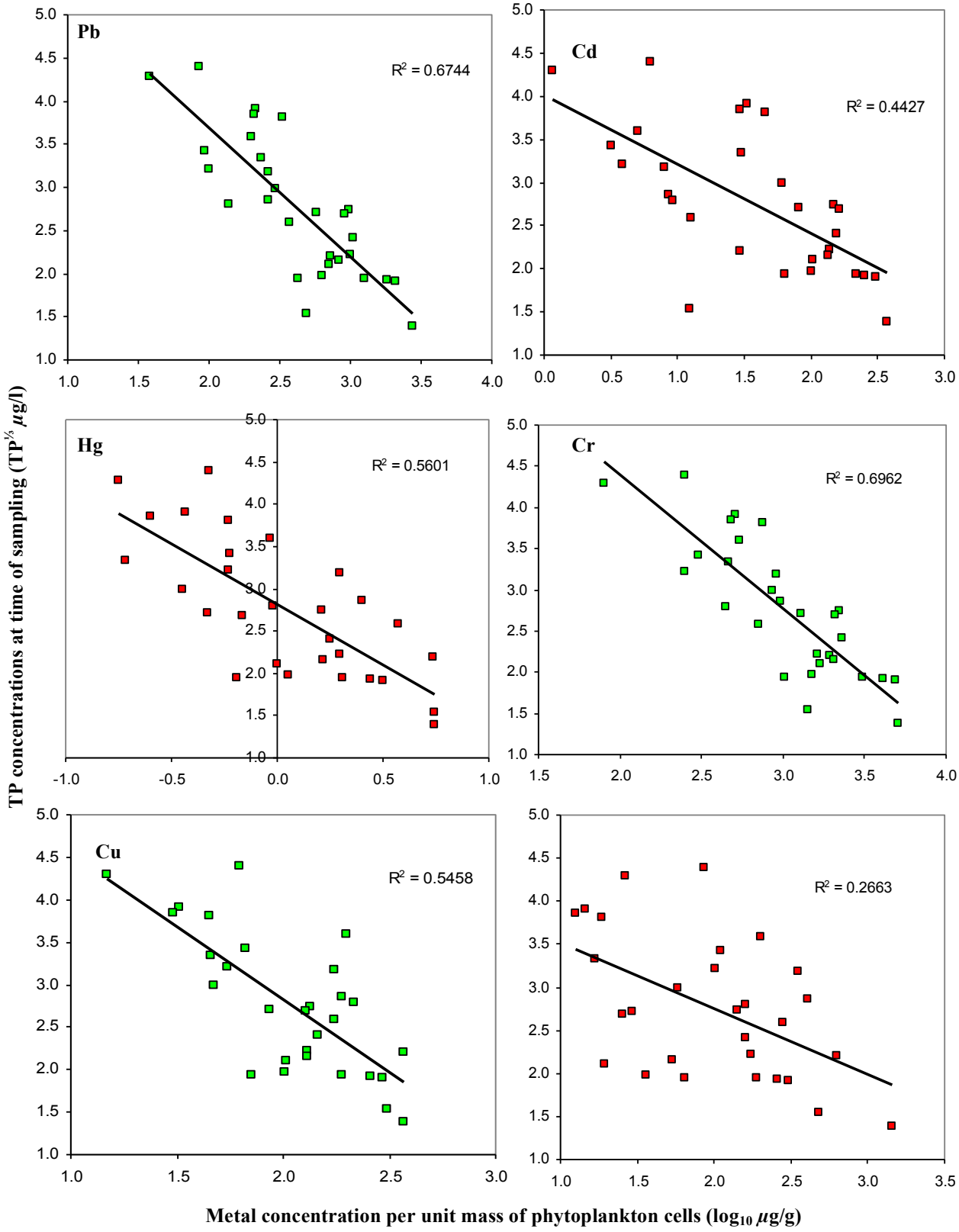


Figure 3. Correlation between Pb, Cd, Hg, Cr, Cu and Ni concentrations per unit mass of phytoplankton and TP concentrations. The data was collected from the samples of all three lakes during each sampling occasion (n=*29*).

A bivariate correlation and regression analysis was carried out on the data in Figure 3 using the Statistical Package for Social Science (SPSS). The correlation coefficient and *p*-values of the tests confirms the patterns in the scatterplot that a significant negative relationship exists between TP and Pb (r = -0.823, $p = 0.00$), Hg (r = -0.741, $p = 0.01$), Cu (r = -0.748, $p = 0.00$), Cd (r = -0.662, $p = 0.00$), Cr (r = -0.837, $p = 0.00$) and Ni (r = -0.532, $p = 0.02$) per unit mass of phytoplankton in the lakes.

In contrast to Pb, Cd, Hg, Cu, Cr and Ni, Co, Mn and P per unit mass of phytoplankton cells showed no clear relationship against the TP concentrations of the three lakes on all sampling occasions. Examination of the bivariate correlation between the variables indicated no significant relationship exists. Due to the extensive number of outliers and the lack of significant correlation between the two sets of variables, a regression analysis was not suitable for the data.

Table 3 summarises the results of the multiple regressions carried out using a combination of chlorophyll-*a* and TP (as the independent variables) against metal (Pb, Cd, Cr, Hg, Cu, Mn, Co) to P ratios per unit mass of phytoplankton cells (the dependant variable). An examination of the *t*-values in Table 3 indicates that TP is a significant predictor of the variations in Pb:P, Cd:P and Cr:P ratios in cells at the 5% level, but chlorophyll *a* alone is not. For the Hg:P ratio in cells, TP is a significant predictor at the 10 % level, but chlorophyll-*a* alone is not a significant predictor.

Table 3. Summary of the simultaneous multiple regression performed using chlorophyll-*a* and total phosphorus (TP) as independent variables and the metal (Pb, Cd, Cr, Hg, Cu, Mn, Co) to P ratios in phytoplankton cells from the three lakes as the dependant variable. Where $p < 0.05$, the relationship was significant at the 5 % level, and where $p < 0.10$, the relationship is significant at the 10 % level.

Metal	Metal : P ratio with			
	Chlorophyll *a*		Total phosphorus	
	t	Sig.	t	Sig.
Pb	-0.474	0.640	-2.541	0.017
Cd	-0.179	0.859	-2.457	0.021
Cr	-0.384	0.704	-2.781	0.010
Hg	-1.018	0.318	-1.710	0.099
Cu	-0.507	0.616	-1.189	0.245
Mn	0.167	0.896	0.683	0.501
Co	-0.635	0.531	0.187	0.853

The relationships in Table 3 are illustrated in Figure 4. This shows the strongest correlation to exist between the Cr:P ratio in cells and TP ($r^2 = 0.3362$).

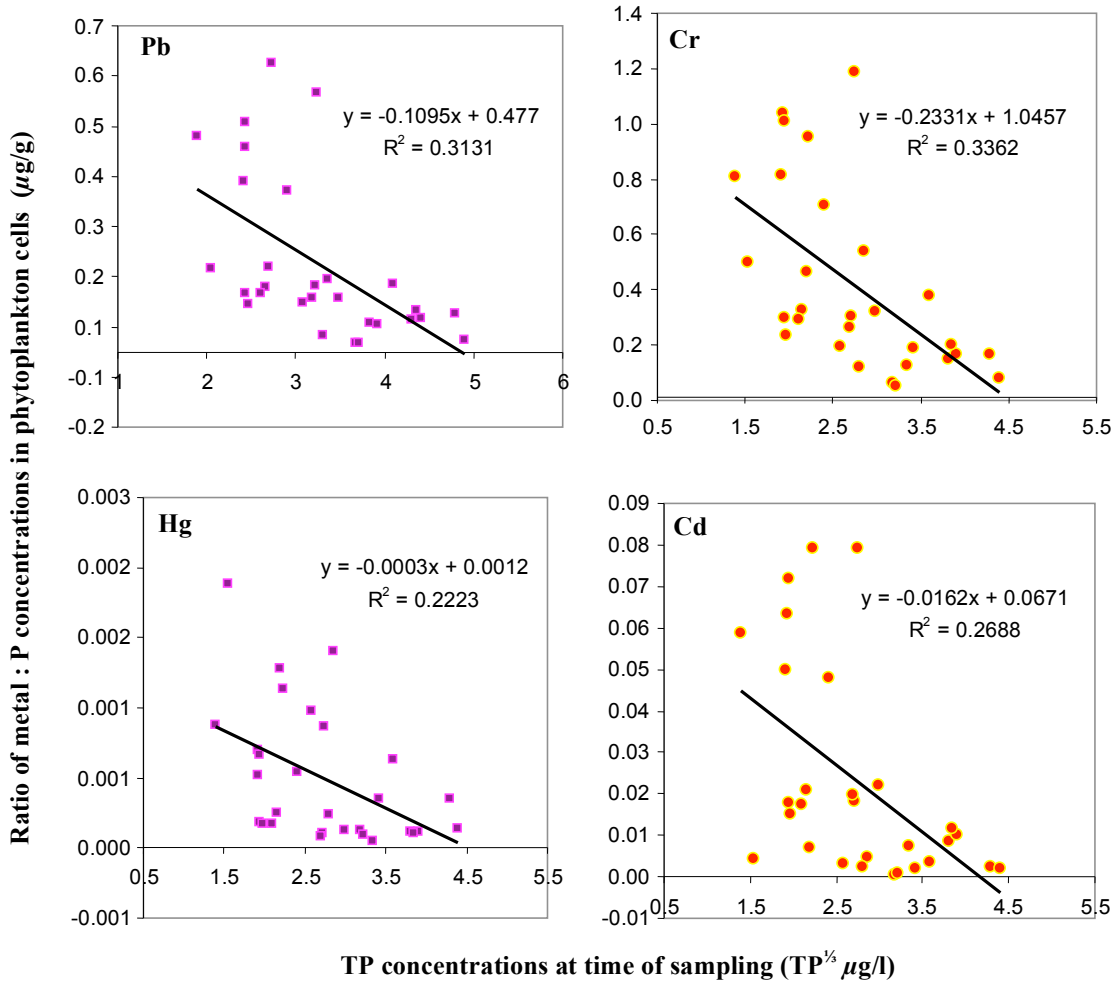


Figure 4. The relationship between TP and metal (Pb, Hg, Cd, Cr) to P ratios per unit mass of phytoplankton cells in the three lakes. As a single variable in the multiple regression between the metal:P ratios against chlorophyll-*a* and TP, TP is a significant predictor of Pb, Cd and Cr: P ratios at the 5 % level, and of Hg: P at the 10 % level (Table 3). Chlorophyll *a* however showed no significant correlation.

Figure 5 shows the dominant groups of phytoplankton (as a percentage of the total volume), illustrating the shifts in species association of the phytoplankton over the sampling period. Among these, the dominant groups in Loch Coire nan Arr (Figure 5.A) were the Chlorophytes (particularly *Cosmarium* sp.) and the Dinoflagellates (particularly *Peridinium*

willei). In Loch Doilet (Figure 5.B), the Chlorophytes were also a dominant group, particularly the filament *Oedogonium* sp. In contrast, Loch Urr (Figure 5.C) had a greater abundance of the blue-green algae, such as the genus *Oscillatoria* sp., which is from the prokaryotic group the Cyanophytes. There was also a higher dominance of the Diatoms in Loch Urr in comparison to the other lakes.

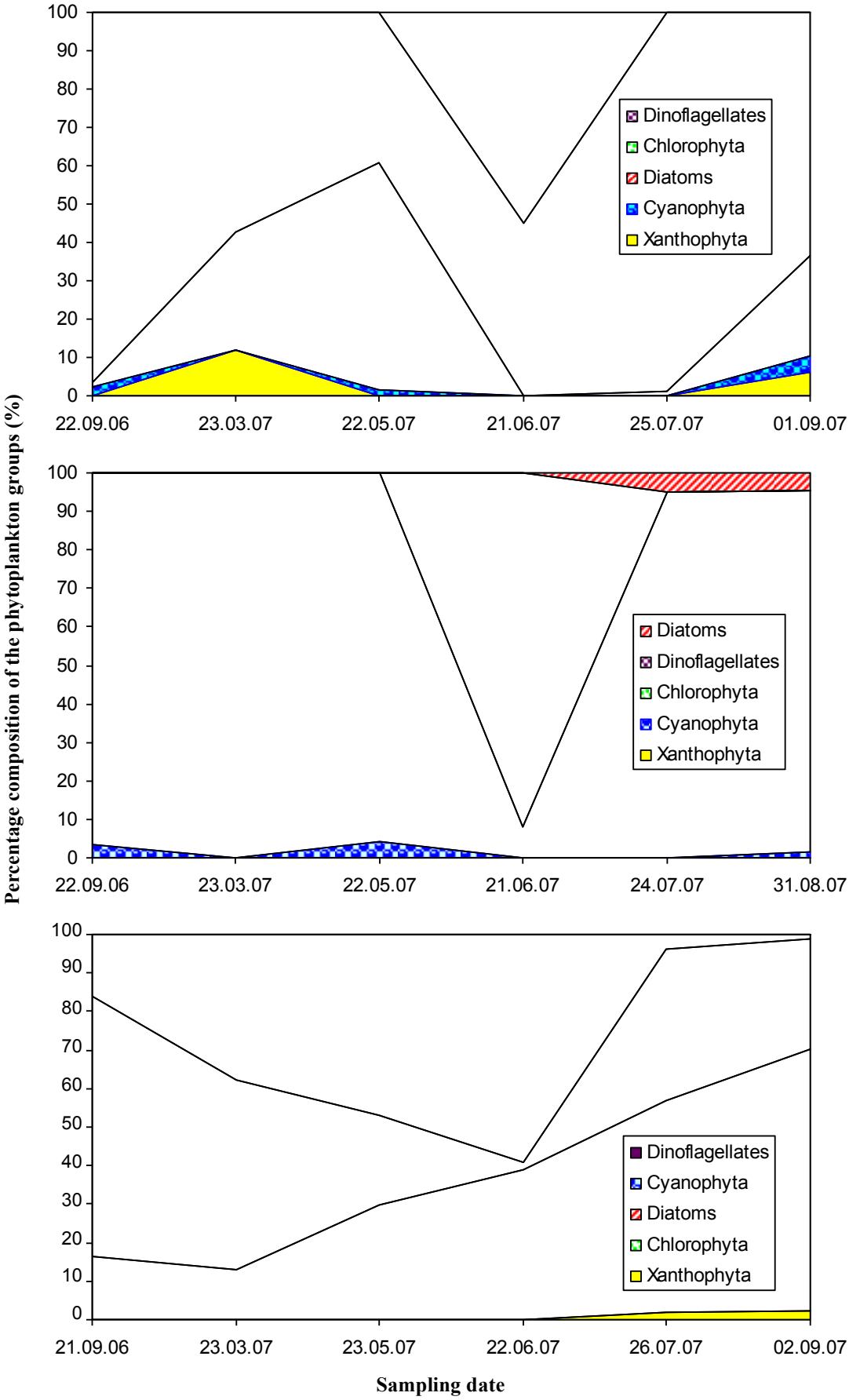

Percentage composition of the phytoplankton groups (%)
100
90
80
70
60
50
40
30
20
10
0
22.09.06
23.03.07
22.05.07
21.06.07
25.07.07
01.09.07
Dinoflagellates
Chlorophyta
Diatoms
Cyanophyta
Xanthophyta
24.07.07
31.08.07
21.09.06
23.05.07
22.06.07
26.07.07
02.09.07
Sampling date

Figure 5. The dominant groups of phytoplankton (as a percentage of the total volume), showing the shifts in species association of the phytoplankton through the sampling period. The percentage composition if presented for Loch Coire nan Arr **(A)**, Loch Doilet **(B)** and Loch Urr **(C).**

The data for the total number of cells per ml, and their total surface area and volume biomass for each sampling occasion in the three lakes are presented in Figure 6. In some cases the patterns have similar timings in their fluctuations. The cases where an inverse relationship between cell count and surface area or biomass, for example in Loch Doilet on the 23/03/07, can be attributed to a decline in cell number but not in the specific cell size during that period. The maximum biomass, surface area and cell count calculated for Loch Coire nan Arr were respectively 77.5 μg/l, 23.1 mm^2/l 52.5 cells/ml on the 26/06/07. In Loch Doilet, the maximum recorded were 35.6 μg/l for biomass, 34.6 mm^2/l for surface area and 31.7 cells/ml for the total cell count on the 01/09/07. Loch Urr held a maximum biomass of 445.6 μg/l on the 01/09/07, surface areas of 9278.3 mm^2/l on the 26/07/07, and cell count of 307.6 on the 26/07/07.

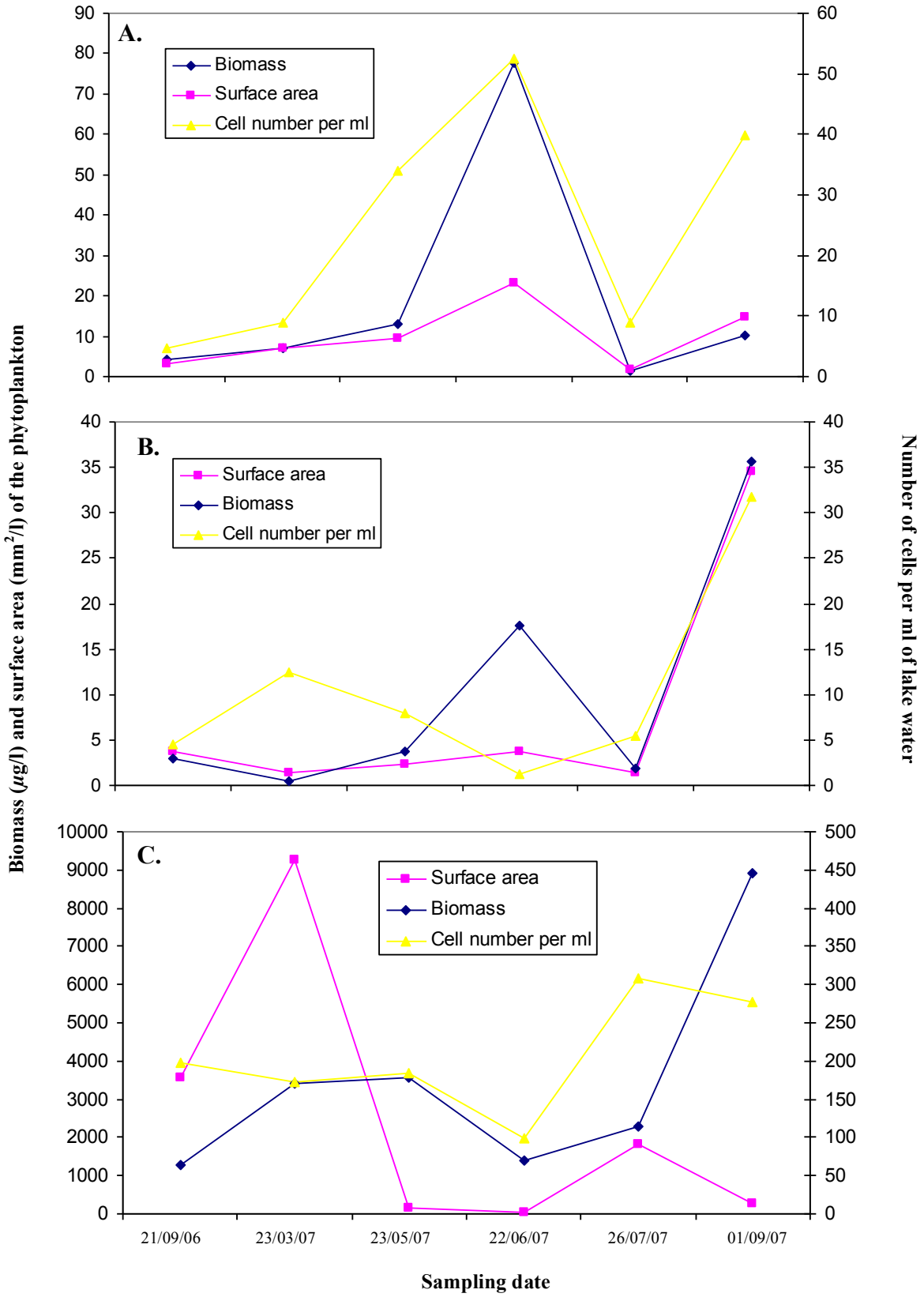


Figure 6. The total number of cells per ml, and their total surface area and volume biomass for each sampling occasion in Loch Coire nan Arr **(A)**, Loch Doilet **(B)** and Loch Urr **(C)**.

The values are based on the sum of the values for each phytoplankton identified. The series key provided in the charts applies to each of the trend lines.

Figure 7 shows the correlation between the surface area, total biomass and cell count data and the TP concentrations determined for the time of sampling. The significance of the regression line calculated with SPSS is displayed below the r^2 value on each chart. These indicate that the correlation between the number of cells per ml and TP is significant at the 5 % level. The relationship between cell volume biomass and TP is significant at the 10 % level, while the relationship between surface area and TP shows no significance.

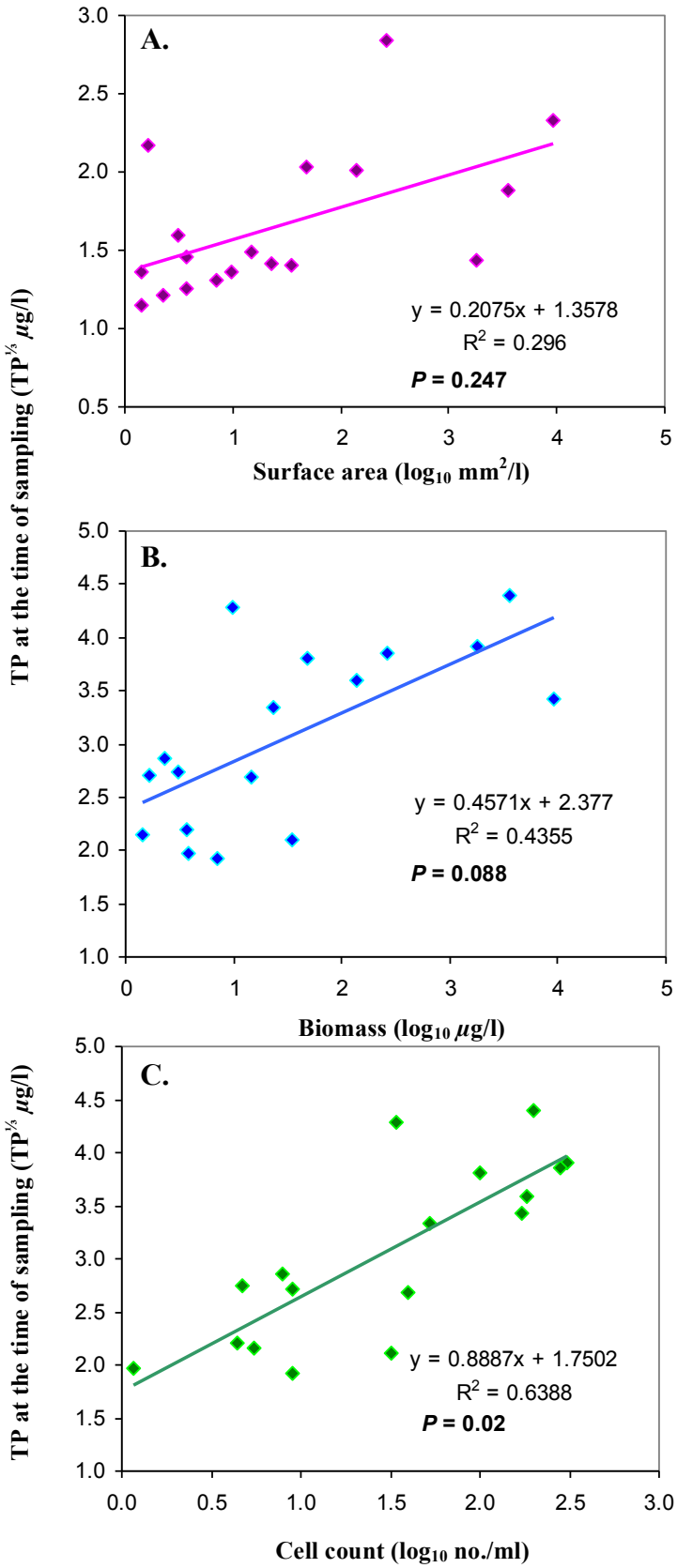


Figure 7. Correlation between the surface area **(A)**, total biomass **(B)** and cell count **(C)** data against the TP concentrations determined for the time of sampling from early June 2006 to late September 2007 in all three lakes. The significance (*p*) values were computed with SPSS on the significance of the regression line. These show C to be significant at the 5 % level, B at the 10 % level, and A to not be significant.

The regression models obtained for TP and cell count (Figure 7 C), and those generated for cell count and the concentration of metals per gram of cells (Eq. 1-8) were used to calculate the best fit values that describe the effect of changes in cell density on metal uptake by the phytoplankton under different trophic states.

This was completed by firstly using the regression equation for TP and cell count (Figure 7 C) to estimate the number of cells per ml under a range of TP concentrations. These data were then incorporated into the following regression equations obtained from the analysis of the metals and P per unit mass of phytoplankton and the corresponding cell count.

$$Pb = ((-1.888 \mathbf{x} \log_{10}(\text{cell count}) + 9.973)^3 \quad \text{Eq. 1}$$

$$Hg = ((-0.268 \mathbf{x} \log_{10}(\text{cell count}) + 1.354)^3 \quad \text{Eq. 2}$$

$$Cu = ((-0.874 \mathbf{x} \log_{10}(\text{cell count}) + 5.813)^3 \quad \text{Eq. 3}$$

$$Cd = ((-1.006 \mathbf{x} \log_{10}(\text{cell count}) + 4.864)^3 \quad \text{Eq. 4}$$

$$Cr = ((-2.530 \mathbf{x} \log_{10}(\text{cell count}) + 13.412)^3 \quad \text{Eq. 5}$$

$$Co = ((-0.538 \mathbf{x} \log_{10}(\text{cell count}) + 2.572)^3 \quad \text{Eq. 6}$$

$$Mn = ((-0.967 \mathbf{x} \log_{10}(\text{cell count}) + 10.609)^3 \quad \text{Eq. 7}$$

$$P = ((-1.114 \mathbf{x} \log_{10}(\text{cell count}) +16.551)^3 \quad \text{Eq. 8}$$

This generated best-fit values for each metal per gram of cells. For example, the Hg per gram of phytoplankton in water with a TP concentration of 30 μg/l was calculated as follows:

- **Phytoplankton cells per ml:**

  $= 10 \wedge ((30^{1/3} - 1.7502) / 0.8887)$

= 33.3 cells

- **Hg per gram of phytoplankton:**

$= ((-0.268 \times \log(33.3)) + 1.354)^3$

$= 0.85\ \mu g/g$

Using the same regression equation for TP and cell count (Figure 7 C), a TP of 145 μg/l yields a cell count of 8558 cells per ml. The regression equation for predicting the Hg concentration per gram of cells based on cell count (Eq. 2) then gives an estimate of 0.03 μg of Hg per gram of cells. Table 4 provides details on how the predicted Hg concentrations change per gram of cells with a range of TP concentrations.

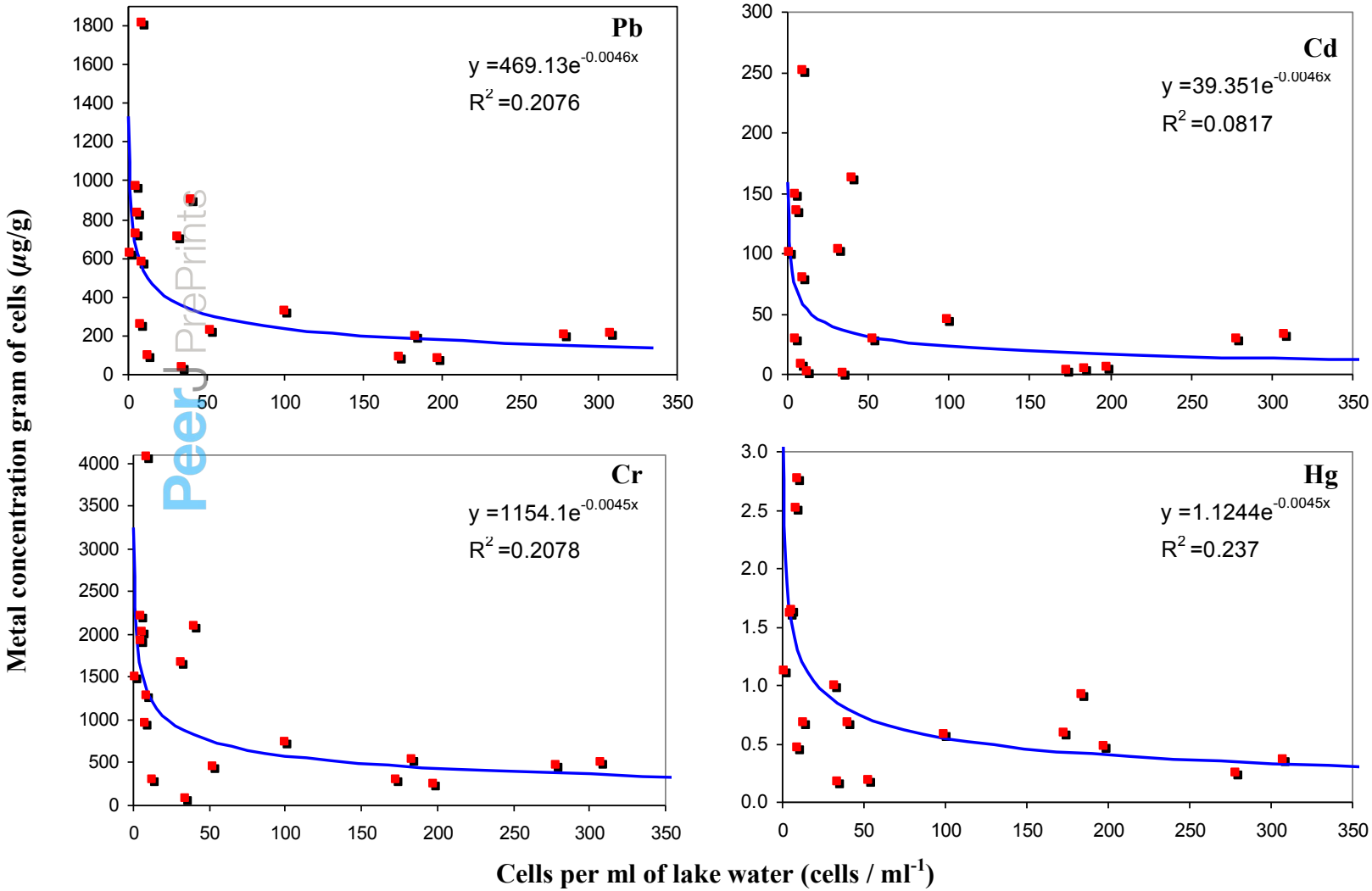
Table 4. Best fit values of the number of phytoplankton cells per ml under a range of trophic states and the concentration of Hg per unit mass of those cells. The cells per ml were predicted using the regression formula generated for TP and cell counts in this study (Figure 4 C). Concentrations of Hg per μg of cells were estimated using the predicted cells per ml and the regression equation for Hg per unit mass of phytoplankton (Eq. 2).

TP (μg/l)	Phytoplankton cells per ml	Hg per unit mass of cells (μg/g)
10	2.84	1.87
12	4.02	1.69
14	5.50	1.54
16	7.30	1.41
18	9.48	1.30
20	12.08	1.21
22	15.15	1.12
24	18.75	1.04
26	22.94	0.97
28	27.78	0.91
30	33.34	0.85

Figure 8 shows the best fit lines for the relationship of cell counts and the concentration of Hg, Cd, Cr, Cu, Co, Mn, Ni and Pb per gram of cells. These were calculated in the same way as described in detail for Hg, with an extension of that data to include the range of TP values recorded in this study (7-85 μg/l). As the best fit curves are without noise, and because they represent the correlations in the data obtained from this study, they can be used to examine the rate of metal uptake by phytoplankton cells in this study. The data points, i.e. the true measurements recorded, were used in an exponential regression to quantitatively describe the rate of uptake by the phytoplankton.

The best fit lines in Figure 8 suggest that the uptake of Hg, Pb, Cd, Cu, Co, Ni and Cr by the phytoplankton is subject to exponential decay. This is characterised by an initially rapid decline in metal concentrations per μg of phytoplankton with increasing cells, until the concentration approaches zero, where the rate of the absolute decrease in the metals decelerates. The exponential regression equations for the data points in Figure 8 shows the decay constant, which defines the rate of metal decay in phytoplankton cells with an increasing number of cells. The larger the rate constant, the more rapid the decay of the dependant variable (y, metals in phytoplankton). The rate of Pb, Cd, Cr, Hg, Cu, Co, Ni and Mn decay in phytoplankton cells with an increasing number of cells is 0.0046, 0.0046, 0.0045, 0.0045, 0.0037, 0.0069, 0.004 and 0.0031 (mL/cell) respectively.

453



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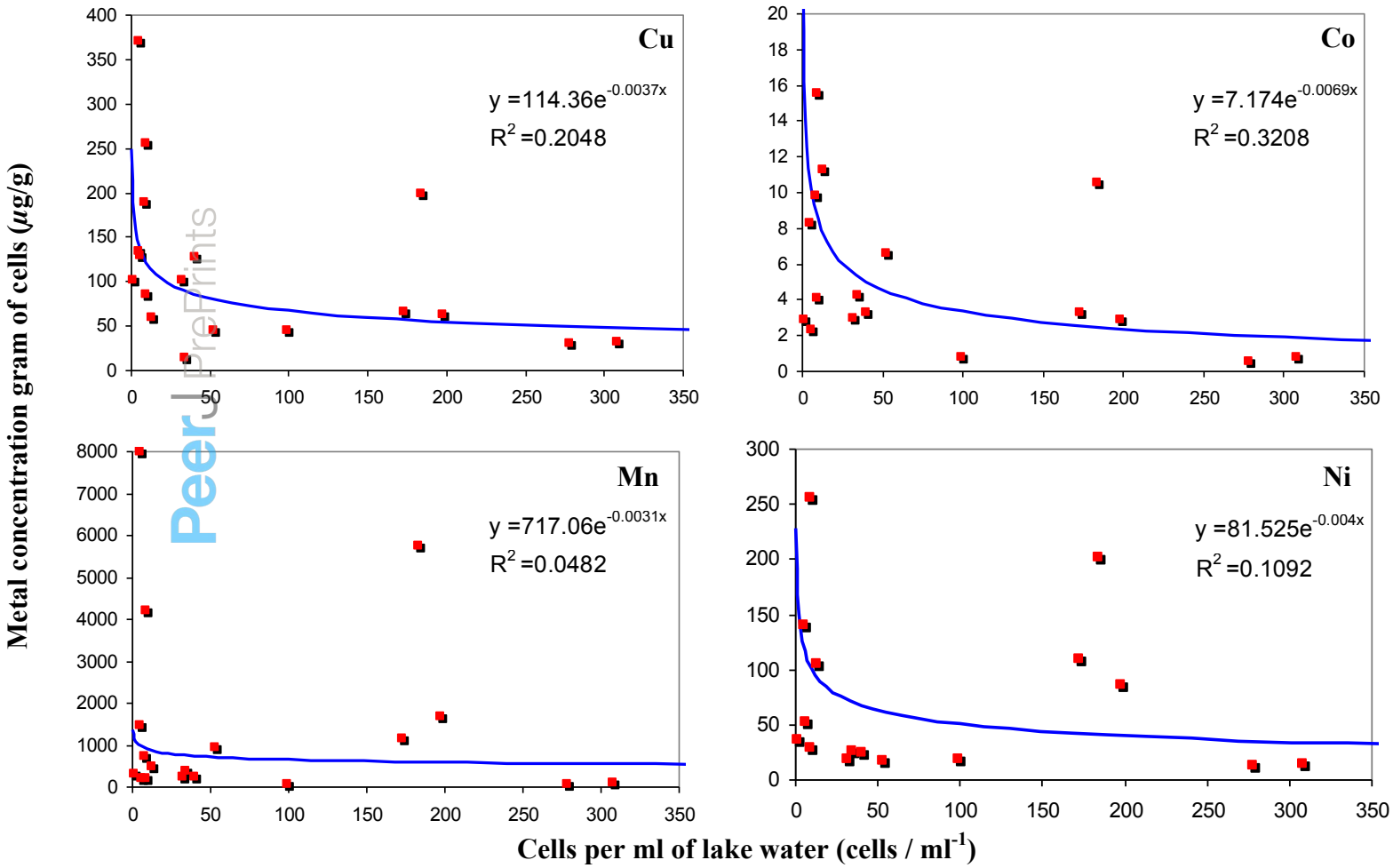

Figure 8. The relationship of phytoplankton cell counts with Pb, Cd, Co, Cu, Cr, Mn, Ni and Hg per gram of cells. The best fit lines (in blue) were calculated from the predicted cell counts (Figure 4.9.C) and the metal (and P) concentrations per unit mass of cells (Figure 4). The data points are the actual measurements recorded in this study and were used in the exponential regression of the formula displayed for each relationship.

As an additional observation, Figure 9 shows the line of best fit for TP and phytoplankton cell count. This was calculated with the regression models obtained for TP and cell count (Figure 7 C). The data points are the actual measurements recorded, and were used for the exponential regression analysis displayed to quantitatively describe the growth of cells in response to rising TP conditions. Figure 9 suggests that cell production with increased TP concentrations is subject to exponential growth. This is characterised by an initial gradual rise in cell count with increasing TP, but as more TP is introduced, the rate of growth accelerates.

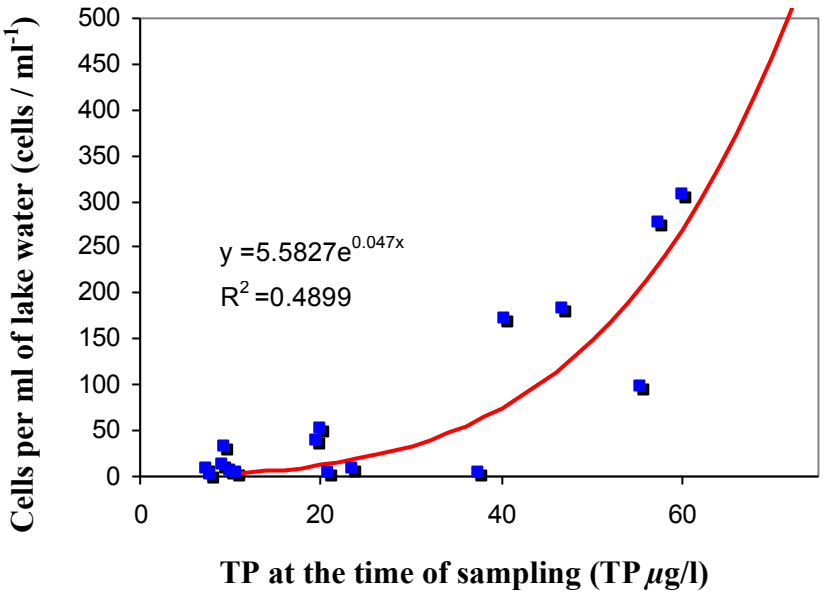

Figure 9. The relationship of phytoplankton cell counts with TP concentrations. The best fit line was calculated from the regression analysis of TP and cell counts (Figure 8 C). The data points are the actual measurements recorded in this study and were used in the exponential regression of the formula displayed.

The metal concentrations in one cell of phytoplankton were calculated by firstly calculating the weight of an individual cell. For example, in Loch Doilet on the 23/05/2007 the phytoplankton cell count was 7.95 cells/ml and the mean phytoplankton biomass was 3.77 μg/l. Therefore the weight of one cell is calculated as follows.

- **Phytoplankton cell biomass (μg/l) ÷ number of cells per litre (cells/l)**

= 3.77 μg/l ÷ 7950 cells/l

= 4.74 x 10^{-4} μg (mean weight of one cell in Loch Doilet)

Secondly, the concentration of metals was calculated for one cell. This was carried out by using the weight of one cell and the concentration of metal per unit weight of cells. The above cell weight for Loch Doilet on the 23/03/2007 and the concentration of Cd per gram of cells will be used as an example here.

- **Weight of individual cell (g/cell) x Cd per gram of cells (μg/g)**

= 4.74 x 10^{-10} (g/cell) x 8.5 (μg/g)

= 4.03 x 10^{-15} g of Cd per cell

Table 5 shows the calculated concentrations for Hg, Pb, Cd, Cu, Cr, Co, P, Mn and Ni in the phytoplankton cells of each of the lakes on all sampling occasions. The average concentration of the metals per cell were 84.01 g x 10^{-14} (Pb), 12.41 g x 10^{-14} (Cd), 1.68 g x 10^{-15} (Hg), 200.01 g x 10^{-14} (Cr), 136.21 g x 10^{-15} (Mn), 15.76 g x 10^{-14} (Cu), 95.21 g x 10^{-16} (Co), 7.79 g x 10^{-9} (P) and 10.79 g x 10^{-14} (Ni).

Table 5. Content of Pb, Cd, Hg, Cr, Co, Ni, Mn, P and Cu per phytoplankton cell in the three lakes on all sampling occasions. The values were calculated from the average weight of one cell, and the metal (and P) concentrations per gram of cell on the same date.

Lake	Date	Metal content per phytoplankton cell								
		Pb ($g \times 10^{-15}$)	Cd ($g \times 10^{-15}$)	Hg ($g \times 10^{-15}$)	Cr ($g \times 10^{-14}$)	Co ($g \times 10^{-16}$)	Ni ($g \times 10^{-14}$)	Mn ($g \times 10^{-14}$)	P ($g \times 10^{-12}$)	Cu ($g \times 10^{-14}$)
Loch Coire nan Arr	22.09.06	906.6	139.0	1.5	207.5	77.3	13.2	138.2	1.8	12.6
	23.03.07	1444.3	200.1	2.2	325.3	124.1	20.4	333.6	3.2	20.4
	22.05.07	14.5	0.4	0.1	3.0	16.4	1.0	14.8	0.2	0.6
	21.06.07	343.7	44.4	0.3	68.2	97.5	2.5	137.9	6.0	6.7
	25.07.07	90.2	12.5	0.1	20.0	6.4	0.5	3.3	0.7	1.3
	01.09.07	229.8	41.4	0.2	53.3	8.4	0.7	5.9	2.1	3.3
Loch Doilet	22.09.06	471.3	19.1	3.6	125.9	693.9	40.7	520.8	2.8	24.1
	23.03.07	4.3	0.1	0.0	1.2	4.7	0.4	2.1	0.1	0.3
	22.05.07	124.1	4.0	1.2	45.4	46.5	19.4	34.7	0.9	9.0
	21.06.07	9461.8	1509.8	16.9	2255.6	434.5	54.6	479.5	100.7	152.5
	24.07.07	273.6	44.6	0.5	66.6	7.5	1.7	6.6	2.1	4.3
	31.08.07	797.7	116.5	1.1	189.6	33.8	2.2	27.9	6.8	11.6
Loch Urr	21.09.06	27.5	2.0	0.2	8.0	9.3	2.8	54.1	1.1	2.0
	23.03.07	92.4	3.2	0.6	29.9	32.5	10.8	113.1	1.7	6.5
	23.05.07	195.7	4.9	0.9	52.8	103.0	19.6	559.3	1.4	19.4
	22.06.07	230.4	31.9	0.4	52.1	5.6	1.3	4.1	3.7	3.1
	26.07.07	80.7	12.5	0.1	19.0	3.0	0.5	4.0	1.2	1.2
	02.09.07	333.6	46.9	0.4	76.7	9.6	2.0	11.9	4.0	4.9

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Discussion

As P is a limiting nutrient for phytoplankton growth, TP is a good measure of a lakes trophic status (Brooks *et al*., 2001). From the range (maximum to minimum) of TP concentrations recorded for each lake (Figure 1), the associated trophic status of the lakes ranges from oligio- mesotropic for Loch Doilet (3.7-23.5 μg TP l^{-1}), oligio- eutrophic for Loch Coire nan Arr (2.7-79.3 μg TP l^{-1}), and meso- eutrophic for Loch Urr (22.0-85.3 μg TP l^{-1}). However, the trophic state of a lake is often judged in terms of mean TP concentrations (Carlson, 1977; Knowlton & Jones, 1997; O'Gorman et al., 2004). If the mean TP concentrations over the sampling period are used to assign a trophic status to the lakes in this study, that yields a status of mesotrophic for Loch Coire nan Arr with a mean TP of 22.9 μg/l, oligotrophic for Loch Doilet (9.6 μg TP l^{-1}), and eutrophic for Loch Urr (45.9 μg TP l^{-1}). The variation in the mean trophic state between the three lakes may be partially attributed to several differences in lake and catchment morphometry. For example, Loch Doilet has the lowest mean TP concentration at 9.6 μg TP l^{-1} but has a lake volume (4.2 x 10^6 m^3) that greatly exceeds that of the other two lakes (5.0 x 10^5 m^3 in Loch Coire nan Arr, 2.4 x 10^6 m^3 in Loch Urr). It also has a relatively higher maximum lake depth recorded at approximately 16 m in comparison to a maximum depth of 12 m recorded in the other two lakes (Table 1). A larger lake volume and maximum depth tends to result in lower nutrient concentrations (Chow-Fraser, 1991). This is because firstly, the TP can be diluted by a high volume of lake water, and secondly, at greater lake depths there is less possibility of mixing and therefore P can be more readily removed from the water column by the sediment to the lake bed (Jeppesen *et al*., 2003).

The variations in TP concentrations recorded across the study period often show similar timing in their fluctuations to that of chlorophyll-*a* trends (Figure 1). Also, the predictions of chlorophyll-*a* concentrations by the models of Prairie *et al*. (1989) and the

OECD (1982) show some agreement with the observed values, particularly in Loch Doilet. These relationships are mainly owing to the rise in lake water temperatures during the summer months, when six of the ten sampling occasions took place. Not only does this result in greater evaporation and therefore less dilution of P, but also a rise in the photosynthetic pigment (chlorophyll-*a*). The stimulated growth of phytoplankton causes higher community respiration rates that reduces dissolved oxygen (Mackay & Shiu, 1981). In turn, a redox sensitive release of P from the oxidised surface layer of sediments is instigated, further stimulating the growth of phytoplankton due to the enhanced availability of nutrients. There are however some deviations to these trends, particularly in Loch Coire nan Arr and Loch Doilet during May 2007 where a sudden peak in TP was observed. An influencing factor here is that April 2007 was the warmest April in the British Isles since 1659, and was also very dry and sunny with maximum Scottish temperatures of 17.4°C (Eden, 2007). The resultant increased evaporation and low rainfall may have lowered lake water levels may, making the TP more concentrated. A change in lake water levels was particularly noticeable in Loch Coire nan Arr where the maximum lake depth lowered from 9 m in April 2007 to 3.5 m in May 2007. Although the main factor contributing to such a large change was a nearby fish hatchery that is resourced by the outlet of Loch Coire nan Arr (the Russel Burn River). Due to the dry conditions in April 2007, the company that controls the fish hatchery (Lighthouse Caledonia Ltd.) were forced to construct a dam at the outlet, lest further water was lost from the lake, which would have inhibited smolt production (Henry Dalgety, Lighthouse Caledonia Ltd., personal communication, 2007). The chlorophyll-*a* concentrations appeared to respond to the TP rise in the following months where a sudden peak was observed in early July 2007. Loch Doilet did not exhibit such trends as the chlorophyll-*a* only showed a small increase following the TP peak. This is again possibly due to the greater depth of Loch Doilet, which may be more significant during the calmer weather of April 2007 as the wave disturbance

would be reduced, allowing the TP to be more rapidly removed from the water column than in Loch Coire nan Arr. Another notable deviation in the general relationship of TP and chlorophyll-*a* was in Loch Urr, as illustrated in Figure 1. This can be attributed to a number of factors. Firstly, the timing at which the sampling took place ranged from 9.00 am – 8.00 pm in Loch Urr. As it has been reported that chlorophyll-*a* concentrations are at their highest towards the end of the day (Baars & Oosterhuis, 1982), the variations in time would be expected to cause some fluctuations. Also, an increase in biomass is not always followed by an increase in chlorophyll-*a* concentrations, and samples with the same chlorophyll-*a* concentration do not always have the same biomass because the under-water light conditions influence the chlorophyll-*a* content of phytoplankton (Simon & Helliwell, 1998). Furthermore, the abundance of bacterioplankton (free floating bacterial component of the plankton) is not accounted for in this investigation. As the bacterioplankton have been recognised to compete with algae for P in the water column (Currie, 1990), a rise in TP concentrations in the samples analysed may not be accompanied by a rise in chlorophyll-*a* concentrations in another sample from that same environment.

In a similar context, Figure 6 shows that positive correlations exist between TP and phytoplankton cell count, surface area and biomass. This shows that the strongest relationship was between TP and cell count ($r^2 = 0.6388$), which was significant at the 5 % level ($p = 0.02$). However the correlation with biomass was only significant at the 10 % level ($p = 0.088$) and surface area was not significant ($p \geq 0.1$). A lesser significance in the latter correlation has been previously noted by Thomann (1977) who suggests that the relationship is a combination of biomass, TP, retention time, and sinking rates. It is possible that the three measurements of phytoplankton growth in Figure 6 responded to TP at different rates. For example, count can remain constant even if volume increases, but if the volume per cell declines then the opposite applies, i.e. cell total volume remains constant but the number of

cells increases. Surface area can vary with either, for example a small spherical cell can have a greater surface area to volume ratio than a larger spherical cell. Equally, the variations in the correlations may also be because the method for the determination of cell count is open to less error than that of cell surface area and/or biomass. The latter are an extension of the determination of cell count and their final values include measurements of cell dimensions that fit into an assigned geometric formula. Additionally, Gleskes and Kraay (1983) and Reynolds (1984) shed doubt on the accuracy of the 'classical method' for the quantification of phytoplankton growth. This is because it is based on spot samples that do not account for lateral and vertical fluctuations in lake temperature, nutrients and light availability, as these strongly influence the species composition and abundance of phytoplankton. Phycologists have also recognised that phytoplankton biomass can never be accurately quantified due to diurnal variations (Brian Whitton, personal communication, 2006). Considering the significant relationship between TP and cell count, and that the use of cell count introduces the least error to the final result, it is perhaps more accurate to base interpretations of phytoplankton growth and metal interactions on cell count as opposed to biomass or surface area.

The significant correlations between the mass-specific Pb, Cd, Hg, Cr, Cu and Ni in the phytoplankton and TP concentrations (Figure 3) suggest that algae bloom density dilution occurred in the lakes investigated. This evidence supports the findings of Pickhardt et al. (2002) for algae bloom dilution of Hg. It also relates to studies that have reported algae bloom dilution of As (Chen & Folt, 2000), and polychlorinated biphenyls (Larsson et al., 1992).

Two mechanisms may explain these findings. Firstly is surface availability (Chen and Folt, 2005). This means the phytoplankton share a finite pool of metals and have a constant uptake. Thus enhanced lake productivity reduced the mass-specific metal concentrations. Yet it is difficult to accept that surface availability controlled metal uptake by the phytoplankton

alone because the mass-specific concentrations of Mn showed no correlation with TP (r^2 = 0.0004), while Co (and P) showed no significant decline with increasing TP concentrations. Secondly, because the trace element to macronutrient (i.e. phosphorus or carbon) ratios is a balance of net steady-state uptake and growth rates (Sunda and Huntsman, 1997, 2004). As nutrients become more available, growth rates increase, which eventually results in a decline in element to phosphorus ratios in the cells. The significant correlations ($p < 0.05$) between the mass-specific metal (Pb, Cd, Cr, Hg) to P ratios in phytoplankton and TP (Figure 3), and their negative correlation against chlorophyll-*a* appear to be in agreement with this biodilution hypothesis. This also may explain why Mn showed no correlation with TP. Mn is an essential element for phytoplankton growth (Morel et al., 1991), and so new cells may assimilate the available Mn.

Figure 9 indicates that the relationship of increasing TP and cell count is subject to exponential growth (Serruya & Berman, 1975). Figure 8 suggests the relationship of increasing cell numbers and their Hg, Pb, Cd, Cu, Co, Ni and Cr concentrations follows the pattern of exponential decay. The association between Figure 8 and 9 not only provides potential insight into the rate at which algae bloom dilution occurs. That is, as TP increases, phytoplankton cell growth accelerates gently, and the concentration of metals in cells rapidly decline until it approaches zero, where the rate of the absolute decrease in the metals reduces. This deceleration in algae bloom dilution may eventually be paralleled by a lack of P to sustain the growth of more phytoplankton or insufficient growth space.

The exponential relationships in Figure 8 also suggest that the selective uptake of metals by the phytoplankton occurred (Santana-Casiano et al., 1995). If the decay constants in Figure 8 are examined, it is evident that the rate of Pb decay in phytoplankton with increasing cell number is more rapid than Cu with respective decay constants of 0.0046 and 0.0037. It is also evident that algae bloom dilution is least effective on the most essential metal Mn with a

decay constant of 0.0031. The differences in the rate constants of the algae bloom dilution suggest the involvement of two intracellular mechanisms in the selective uptake of metals. One is metabolic, which attempts to sustain the essential metals (e.g. Mn) concentrations (Sunda and Huntsman, 1998). The other is a detoxification process that stores excess P as intracellular polyphosphate, which protects the cells by binding with metals in a detoxified form (Walsh and Hunter, 1995). If the correlation between the ratios of metals to P in cells with TP in this study (Figure 3) is consulted again, it is notable that the only metals that showed a significant decrease in their ratio to P were Pb, Cd, Hg and Cr. It is also notable that these four metals had a strikingly similar decay constant with their relationship in phytoplankton to increasing cells. That is, 0.0046 for both Pb and Cd, and 0.0045 for Cr and Hg (Figure 8). Additionally, of the metals tested in this study, these four metals are considered the most toxic to phytoplankton (Xue and Sigg, 1993). Therefore, it is possible that when nutrients became more available, growth rates and cellular P increased, forming intracellular polyphosphate bodies that selected less toxic metals more rapidly.

Table 6 presents the metal to P stoichiometries (mol:mol) of the freshwater phytoplankton collected in this study. The calculations were based on the mean concentrations of the metals per cell in each of the three lakes (Table 5). These were converted to molar concentrations and divided by the sum of all components, which included the C and N molar concentrations based on the standard Redfield (1958) ratio of C_{106}:P_1:N_{16}. Table 4 shows the ratios of the metals between the lakes are in the same order of magnitude. The mean metal to P stoichiometry from this investigation is $(C_{106}P_1N_{16})_{1000}Pb_{0.019}Hg_{0.00004}Cu_{0.013}Cd_{0.005}Cr_{0.2}Co_{0.0008}Mn_{0.2}Ni_{0.012}$. This is similar to the phytoplankton cell stoichiometry presented by Twining et al. (2004) who found, for instance, 0.26 mol of Mn for every 1 mol of P, whereas this study found 0.21 mol of Mn for every 1 mol of P. The slightly higher ratio offered by Twining et al. may be expected as their study

was on marine phytoplankton. This is because P is generally more concentrated in the phytoplankton of freshwater lakes, and thus lowering the metal to P ratio.

Table 6. Metal to P stoichiometries (mol:mol) of the freshwater phytoplankton collected in Loch Coire nan Arr, Loch Doilet and Loch Urr for this study. Calculations were based on the mean concentrations of the metals per cell in the three lakes (Table 3). These were then converted to molar concentrations, and divided by the sum of all components, which included C and N molar concentrations that were calculated based on the standard Redfield (1958) ratio of C_{106}:P_1:N_{16}. The averages of the ratios across the lakes yields a mean metal to P stoichiometry of $(C_{106}P_1N_{16})_{1000}Pb_{0.019}Hg_{0.00004}Cu_{0.013}Cd_{0.005}\ Cr_{0.2}Co_{0.0008}Mn_{0.2}Ni_{0.012.}$

Element	Loch Coire nan Arr	Loch Doilet	Loch Urr	Mean
C	105860	106197	105989	106015
N	15979	16030	15998	16002
P	999	1002	1000	1000
Pb	0.03	0.01	0.01	0.019
Hg	0.00005	0.00003	0.00003	0.00004
Cu	0.02	0.01	0.01	0.013
Cd	0.009	0.004	0.002	0.005
Cr	0.3	0.1	0.1	0.2
Co	0.001	0.001	0.001	0.0008
Mn	0.3	0.1	0.3	0.2
Ni	0.01	0.01	0.01	0.012

The calculated stoichiometry may be used to estimate the concentration of metals per phytoplankton cell in the lakes based on cell size. If the average biomass of one cell is 1.55×10^{-10} g, and using the Cd: P ratio of 0.000005/1, the estimated Cd concentration bound to a cell is 7.76×10^{-16} mol (or 87.2×10^{-15} g). If the P concentration is raised by a factor of 4, the estimated Cd is 3.11×10^{-18} mol (or 3.49×10^{-16} g). The risk of toxicity can then be predicted by comparing the results to those of toxicity tests. For instance, Wang and Dei (2006) observed toxicity at a Cd:P ratio of > 0.2. While this may be useful, using the

stoichiometry as a predictor on a wider scale than the lakes investigated has large uncertainties because it would assume the ratio is constant.

Conclusions

1. A higher trophic status in the lakes resulted in significant algae growth dilution of the mass-specific Pb, Cd, Hg, Cu, Ni and Cr in the phytoplankton. This was because the available metals had to be shared among more and as P became more available, the mass specific metal to P ratios in the phytoplankton declined. The same mechanisms were not effective on Mn because it is assimilated during phytoplankton growth.

2. The relationship between the number of phytoplankton cells per millilitre of lake water and the mass-specific metal concentrations in the phytoplankton provides an examination of the rate of algae bloom dilution in the lakes. As TP increased, phytoplankton cell growth accelerated gradually, and the concentration of metals in cells rapidly declined until it approached zero. The decay constants indicate that Mn has the lengthiest rate of algae bloom dilution among the metals. This suggests the involvement of two intracellular mechanisms in the active selection of metals. The first is metabolic in that growing cells have preference for Mn and thus it is diluted at a more gradual rate. The second is a detoxification process that stores excess P as intracellular polyphosphate, which selects the less toxic metals more rapidly.

3. The significant positive relationship between the concentration of metals per phytoplankton cell and the mass of one cell is consistent with the theory that a cell will obtain the maximum metal diffusion flux depending on the cell diameter.

4. The simultaneous measurements of metals and P in phytoplankton cells, along with quantification of changes in cell mass, generated a mean metal to P stoichiometry of $(C_{106}P_1N_{16})_{1000}Pb_{0.019}Hg_{0.00004}Cu_{0.013}Cd_{0.005}Cr_{0.2}Co_{0.0008}\,Mn_{0.2}Ni_{0.012}$ based on the Redfield

692 average C, N and P stoichiometry of $(CH_2O)_{106}(NH_3)_{16}H_3PO_4$. This stoichiometry can be used
693 to estimate the concentration of metals in cells based on their P content and may be
694 incorporated into BLM if the concentration of cell surfaces were to be used as the biotic
695 ligands.
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897