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METHYLMERCURY UPTAKE AND BIOCONCENTRATION BY THE FRESHWATER GREEN ALGA *Pseudokirchineriella subcapitata*

OGOCHUKWU L. NWOBU

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Abstract

Methylmercury (MeHg) enters most aquatic food webs primarily at the phytoplankton level. However, in the complex aquatic ecosystem, it is difficult to fully examine specific pathways. Consequently, in this study, the uptake of environmentally realistic levels of added MeHg concentrations (0.21 to 20 ng Hg L^{-1}) by the freshwater green alga *Pseudokirchniriella subcapitata* grown in batch and semi-continuous cultures at biomass levels of (0.03, 0.15, 0.3 and 3 mg (dw) L^{-1}) was investigated. In algal culture media without dissolved organic carbon (DOC) and in river water samples with DOC concentration of (2.8 mg L^{-1}) uptake, uptake rate constant, algal concentration of MeHg and the bioconcentration factor (BCF) after 48 h was measured. MeHg uptake was biphasic, with rapid uptake of at least 50% of the total uptake taking place over the first 2 h of incubation followed by a more gradual uptake for the remaining 46 h. Desorption of MeHg from algal cells by re-suspension in acidic medium (pH 3) was < 10% of the total uptake at 48 h. Uptake rate constants of MeHg (k_u) increased with aqueous MeHg concentration ($r^2 = 0.99$). At 48 h, algal concentration of MeHg (MeHg (ng) divided by dry weight of algal biomass (g)) increased with aqueous MeHg concentration ($r^2 = 0.98$) but decreased with increasing biomass ($r^2 = 0.75$). This was shown to be a biomass dilution effect as the total uptake of MeHg did increase with biomass. Log MeHg BCF decreased with increasing algal biomass ($r^2 = 0.99$) with values of 6.9, 6.2 and 5.2 at 0.03, 0.3 and 3 mg (dw) L^{-1} respectively at all concentrations of MeHg added (0.5, 1.0 and 20 ng L^{-1}).

Résumé

Le méthyle de mercure MeHg entre dans la plupart des chaînes alimentaires aquatiques au niveau du phytoplancton. Cependant, comme l'écosystème aquatique est très complexe, il est difficile d'examiner complètement les voies spécifiques. Ainsi, dans cette étude, la prise en charge du MeHg, à des concentrations correspondant à des niveaux environnementaux réalistes (0,21 à 20 ng Hg L^{-1}), par l'algue verte d'eau fraîche *Pseudokirchniriella subcapitata*, (maintenue en cultures de masses et en cultures semi-continues à des biomasses de 0,03, 0,15, 0,3 et 3 mg (dw) L^{-1}) a été étudiée.

La prise en charge, le taux de réaction, la concentration du MeHg dans les algues et le facteur de bioconcentration (FBC) après 48 h ont été mesurés dans les milieux de culture pour algues sans carbone organique dissous (COD) et dans les échantillons d'eau de rivière avec des concentrations de COD de 2,8 et 26 mg L^{-1}. La prise encharge du MeHg était biphasique avec 50% de totale se produisant dans la prise encharge les deux premières heures d'incubation, suivie par une absorbtion plus graduelle lors des 46 h restantes. La désorption du MeHg ions de la resuspension dans un milieu acide (pH 3) était <10% de la prise encharge totale après 48 h. Le taux de prise encharge constant du MeHg k_u a augmenté avec la concentration aqueuse de MeHg ($r^2 = 0.99$). Après 48 h, la concentration du MeHg dans les algues (MeHg (ng) divisé par le poids sec de la biomasse des algues (g)) a augmenté avec la concentration aqueuse de MeHg ($r^2 = 0.98$) mais diminué avec l'augmentation de la biomasse ($r^2 = 0.75$). Ceci démontre un effect de dilution par la biomasse, puisque la prise enchange totale du MeHg augmente avec la

biomasse. Le log MeHg FBC a diminué avec l'augmentation de la biomasse des algues ($r^2 = 0.99$) avec des valeurs de 6,9, 6,2 et 5,2 à 0,03, 0,3 et 3 mg (dw) L^{-1} pour toutes les concentrations de MeHg ajoutées (0,5, 1,0 et 20 ng L^{-1}).

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Table of Contents

Abstract and Resume	i -i ii
Acknowledgments	iv
Table of Contents	v-vii
List of Tables	viii
List of Figures	ix-x
List of Abbreviations	xi

Chapter 1 Introduction		**1**
1.1	Rationale for thesis	1
1.2	Objectives of study	2
1.3	Hypotheses tested	3
1.4	Hg in the Environment	3
1.5	Environmental fate of MeHg	4
1.6	Interactions of MeHg complexes with algae	6
1.6.1	Uptake	6
1.6.2	Bioconcentration	6
1.6.3	Mechanism of MeHg transport into algae	7
1.6.3.1	Passive diffusion	8
1.6.3.2	Facilitated diffusion	8
1.6.3.3	Active transport	9
1.7	Bioconcentration Factor BCF	9
1.8	Previous studies	10
1.9	Factors affecting uptake and algal concentration of MeHg	13
Chapter 2 Methods		**16**
2.1	Algal Cultures	16
2.1.2	Batch culture	17
2.1.3	Semi-continuous culture	18
2.2	Harvesting of algal cells	19
2.3	Algal biomass	21
2.4	Uptake experiments	22
2.4.1	Incubation of cells in artificial medium	22
2.4.2	MeHg analysis of Filtrate	24
2.4.3	Desorption test	25
2.4.4	Algal digestion	25
2.4.5	Uptake experiments in high- and low-DOC natural waters	26
2.5	Calculations	27
2.5.1	Calculation of MeHg uptake by algae	27
2.5.2	Estimation of uptake rate constant	27
2.5.3	Calculation of algal concentration of MeHg	28

2.5.4 Calculation of algal dry weight 28
2.5.5 Determination of MeHg BCF in algae 29
2.5.6 Statistical analysis 29

Chapter 3 Results **30**

3.1 Time course of MeHg uptake 30
3.1.1 Uptake experiment using batch cultures 30
3.1.1.1 Effect of growth phase on MeHg uptake 31
3.1.1.2 Effect of MeHg concentration on MeHg uptake in artificial medium 32
3.1.1.3 Effect of algal biomass on MeHg uptake in artificial medium 35
3.1.1.4 Effect of high- and low-DOC natural waters on MeHg uptake 35
3.1.2 Semi-continuous culture (pseudo-steady state) uptake experiments 37
3.2 Desorption test 39
3.3 Growth test 40
3.4 Effects of environmental variables on algal concentration of MeHg 41
3.5 BCF 41

Chapter 4 Discussion and Conclusions **76**

4.1 Time course of MeHg uptake by algae 76
4.2 Factors affecting uptake and bioconcentration in algae 78
4.2.1 Aqueous MeHg concentration 79
4.2.1.1 Percent Uptake 79
4.2.1.2 Uptake and desorption rate constants 80
4.2.1.3 Algal concentration of MeHg 81
4.2.1.4 BCF 82
4.2.2 Algal biomass 82
4.2.2.1 Uptake 82
4.2.2.2 Uptake rate constant of MeHg 83
4.2.2.3 Algal concentration of MeHg 83
4.2.2.4 BCF 84
4.2.3 DOC 84
4.2.3.1 Total uptake 85
4.2.3.2 Uptake rate constant 86
4.2.3.3 Concentration of MeHg in algae 86
4.2.3.4 BCF 87
4.2.4 Growth phase 87
4.2.4.1 Total uptake 87
4.2.4.2 Uptake rate constant 87

4.2.4.3 Comparison of uptake and concentration of MeHg in algal cells grown in batch and semi-continuous culture 88
4.2.3 Conclusions 89

References **92**
Appendices a-e

List of Tables

Table 1. Characteristics of the algal suspensions in MGM, SLR and RR showing algal biomass (mg (dw) L^{-1}), cell estimate (cells mL^{-1}) and the natural habitat that is simulated. 43

Table 2. Quality control test for recovery, accuracy and precision. 44

Table 3. Comparison of total uptake of MeHg by exponential phase vs. stationary phase algal cells over 24 h exposure period. 45

Table 4. MeHg uptake from aqueous MeHg concentrations ranging from 0.21 to 20 ng MeHg L^{-1} by 0.03, 0.3 and 3 mg (dw) L^{-1} algal biomasses over 48 h exposure. 46

Table 5. MeHg uptake rate constant (h^{-1}) of MeHg over 48 h incubation periods in 0.03, 0.3 and 3 mg (dw) L^{-1} algal biomasses in suspensions of different aqueous MeHg concentrations. 47

Table 6. Algal concentration of MeHg after 48 h incubation period in 0.03, 0.3 and 3 mg (dw) L^{-1} algal biomasses in suspensions of different aqueous MeHg concentration. 48

Table 7. Log BCF of MeHg after 48 h in 0.03, 0.3 and 3 mg (dw) L^{-1} algal biomasses in different algal suspensions determined by the equilibrium method. 49

Table 8. MeHg uptake rateconstant (h^{-1}) in 0.03, 0.3 and 3 mg L^{-1} algal biomasses of cells in pseudo steady state conditions over 48 h exposure period. 50

Table 9. Algal concentration of MeHg (ng g^{-1}) in 0.03, 0.15, 0.3 and 3 mg L^{-1} algal biomasses of cells in pseudo steady state conditions over 48h exposure period.51

List of Figures

Figure 1. Filtrate MeHg concentration (ng L^{-1}) over 24 h exposure period of a medium with 3 mg (dw) L-1 algal biomass of exponentially grown algal cells and initially spiked to 72 ng L-1 MeHg. 52

Figure 2. (a) Uptake of MeHg (72 ng L^{-1}) by algae over the first 2 h of exposure. (b) Uptake of the same MeHg concentration over 24 h exposures. 54

Figure 3. Uptake in 72 ng L^{-1} MeHg suspensions by (a) 0.03,(b) 0.3 and (c) 3 mg (dw) L^{-1} algal biomasses of cells in exponential vs. stationary phases. 56

Figure 4. Change in MeHg concentration in (a) 0.03, (b) 0.3 and (c) 3 mg (dw) L^{-1} algal biomass-MGM suspensions initially containing 0.5 ng L^{-1} MeHg over 48 h incubation period. 58

Figure 5. (a) MeHg uptake in 0.5 ng L^{-1} MeHg MGM suspensions containing 0.03, 0.3 and 3 mg L^{-1} algal biomass of *Pseudokirchineriella subcapitata* over 2 h exposure. (b) Same uptake over 48 hr exposure. 60

Figure 6. Change in MeHg concentration in filtrate of suspensions with an initial concentration of 1.0 ng MeHg L^{-1}over 48 h incubation of (a) 0.03, (b) 0.3 and (c) 3 mg (dw) L^{-1} algal biomasses. 62

Figure 7. (a) Uptake in 1 ng L^{-1} MeHg suspension by 0.03, 0.3and3 mg (dw) L^{-1} algal biomasses of *Pseudokirchineriella subcapitata* over 2 h exposure. (b) Same uptake over 48 h exposure time. 64

Figure 8. a) Change in MeHg concentration of St. Lawrence River water sample (0.53 ng L^{-1}) over 48 h of incubation and b) uptake of MeHg by 3 algal biomasses – 0.03, 0.3 and 3 mg L^{-1} over 2 h of exposure. c) Same

uptake as above over 48 h. 66

Figure 9. a) Change in MeHg concentration of Raisin River water sample (0.21 ng L^{-1}) over 48 h incubation by 3 algal biomasses – 0.03, 0.3 and 3 mg L^{-1} over 2 h of exposure b) uptake of MeHg in Raisin River suspension c) same uptake over 48 h. 68

Figure 10. Measured and calculated uptake in 0.5,1.0 and 20.0ng L^{-1} MeHg by 0.03,0.15, 0.3 and 3 mg L^{-1} algal biomasses in suspensions of cells in pseudo steady-state condition over 48 h incubation period. 70

Figure 11. MeHg Desorption from uptakes at different exposure times in 2 h experimental time at (a) 0.5 and (c) 1.0 ng Hg L^{-1} and to 48 h for (b) 0.5 and (d) 1.0 ng MeHg L^{-1}. 72

Figure 12. Chlorophyll-a fluorescence growth checks on algal cells over 48 h. 74

List of Abbreviations

AAP	algal assay procedure medium
BCF	bioconcentration factor
DOC	dissolved organic carbon
DW	dry weight
GC-AFS	gas chromatography atomic fluorescence spectrometry
HDPE	high density polyethylene
MGM	modified growth medium
MINEQL	software for speciation of inorganics
PCB	polychlorinated biphenyls
PS	parent stock of algae
QSU	quinine sulfate units
RR	Raisin River
SLR	St. Lawrence River
THg	total mercury (Hg)

1.0 Introduction

1.1 Rationale for thesis

Bioaccumulation of methylmercury (MeHg) in aquatic ecosystems leading to fish is a major public health concern internationally (UNEP, 2002). Fish consumption is the most important route of human exposure to MeHg bioaccumulation (Hall et al. 1997). Countries like the USA, Canada, Sweden and Finland have guidelines for human consumption of fish that have been established to protect the public from the adverse human health effects associated with chronic exposure to MeHg (Francis et al. 1998; Hakanson et al. 1990). MeHg is a neurotoxin with the brain as its main target. Its deleterious effects are more pronounced in fetuses, infants and children than in adults (EPA, 1997a). Prenatal exposure damages the cellular processes responsible for brain development, causing mental retardation and delayed development. Chronic exposure may result in immune activation that leads to allergy and autoimmune diseases in adults (Stejskal et al. 1996), as well as IQ deficits and abnormal muscle tone in infants (EPA, 1997a).

In an attempt to assess the hazards of MeHg in the environment, the United States Environmental Protection Agency (USEPA) in its latest mercury report to the United States Congress acknowledged a knowledge gap in the "quantitative understanding of the uptake kinetics and bioaccumulation processes of MeHg" at the phytoplankton level (USEPA, 1997a). Understanding this important vector for the introduction of MeHg into the aquatic food web is a key step in predicting MeHg concentrations in fish tissues and

in other high trophic level aquatic organisms consumed by humans and thereby curtailing human poisoning (USEPA, 1997a).

Algae have been found to take up and bioconcentrate MeHg from their surroundings through both metabolism dependent and independent mechanisms (Kirkwood, 1999; Gadd, 1990). Uptake and bioconcentration of MeHg by algae at the base of the food chain constitute the principal and most critical step in the bioconcentration of MeHg in most aquatic ecosystems (Bowles et al. 2001). As primary producers, algae provide most of the energy to support the structure and function of the aquatic food web (Moye et al. 2002). The uptake and bioconcentration of MeHg by algae not only introduces MeHg into the aquatic food chain, but it is then further biomagnified by organisms at each trophic level (Bowles et al., 2001; Hill et al., 1996).

Understanding the details of processes involved in the uptake and bioconcentration of MeHg by algae and determining some of the principal factors that control them is a key step in assessing the environmental and health risks associated with MeHg as a contaminant. In this study, the uptake of environmentally realistic levels of aqueous MeHg concentration by the freshwater green alga *Pseudokirchniriella subcapitata* grown to exponential phase in batch and semi-continuous cultures at varying biomass levels was investigated.

1.2 Objectives of study

The objectives of this study are:

1) to determine the time course for uptake of MeHg by the green alga, *Psuedokirchineriella subcapitata*, using environmentally realistic ranges of MeHg concentration and algal biomass;
2) to determine uptake, uptake rate constant, bioconcentration (algal concentration of MeHg) and BCF in algae after 48 h as a function of MeHg concentration, algal biomass, and DOC concentrations.

1.3 Hypotheses tested

The following null hypotheses (H_o) were tested:

H_{01}: Aqueous MeHg concentration has no effect on uptake, algal concentration and BCF of MeHg by algae.

H_{02}: Algal biomass has no effect on uptake, algal concentration and BCF of MeHg by algae.

H_{03}: DOC has no effect on uptake, algal concentration and BCF of MeHg by algae.

1.4 Hg in the environment

There are three environmentally relevant mercury species: elemental mercury (Hg^o), inorganic (divalent) mercury (Hg^{2+}) and methylmercury (MeHg). Elemental mercury is volatile and is the principal form in the atmosphere. Inorganic mercury is soluble and binds to various organic and inorganic ligands. MeHg is the organic form of mercury found in most aquatic ecosystems and can be generated through biotic and abiotic conversion of the other mercury species (Zhang and Planas, 1994; Hamasaki, 1995).

MeHg can pass through biological membranes and bioconcentrate to high concentrations in algal tissues (Boudou and Ribeyre, 1981; Watras and Bloom, 1992). Due to affinity of MeHg to disulfide (thiol) groups, MeHg bioconcentrates principally in the protein tissues of biota (Rabenstein, 1978; Kerper et al. 1992; Clarkson, 1994).

1.5 Environmental fate of MeHg

In aquatic systems, MeHg has several environmental fates influenced mainly by its complexation reactions with ligands and the redox status of the system:

a) it can be demethylated biotically or abiotically to Hg^{0} and be volatilized back into the atmosphere (Sellers et al., 1996; Marvin-Dipasquale and Oremland, 1998; Gardfeldt et al., 2001),

b) it can sorb to ligands on organic matter in sediments, dissolved organic carbon DOC or suspended organic particulates in the water column (O'Driscoll, 2000), and

c) it can form neutral complexes with available anions such as OH^- and Cl^- (depending on the pH) and be partitioned into algae (Faust, 1992). For example in fresh waters, where pH can vary from < 5 to > 9, MeHg is present as MeHgOH whereas in estuaries and marine systems it forms MeHgCl (Sunda and Huntsman, 1998).

The processes of methylation and demethylation control the level of MeHg in the water column such that detectable MeHg at any time is the net result of these two processes (Berman and Bertha, 1986). In natural, oxic freshwaters, the proportion of MeHg to total Hg (THg) concentration is generally about 0.1 to 5% (Fitzgerald and Mason, 1997;

Watras et al., 1998; Hickey et al. 2005). For example, THg concentrations in 31 lakes in Ontario ranged from 0.8 to 2.1 ng Hg L^{-1} while the MeHg concentration ranged from 0.02 to 0.094 ng Hg L^{-1} (Hickey et al. 2005). In Nova scotia, THg concentrations in 11 lakes ranged from 0.09 to 7.4 ng Hg L^{-1} and the MeHg ranged from 0.028 to 0.255 ng Hg L^{-1} in 9 lakes (Hickey et al. 2005). In anthropogenically affected areas, THg concentrations of 10 to 40 ng Hg L^{-1} or higher have been observed (Domagalski, 1998; Wang et al., 1995).

Mercury compounds have a strong affinity for reduced sulphide, particulate and dissolved organics in the environment (O'Driscoll et al., 2005). Most of the MeHg in any aquatic system is bound to the ligands of dissolved and particulate organic phases in the sediments and the water column (Lindqvist et al., 1991; Meili, 1997; Wang et al. 2005). Aqueous MeHg concentrations are often strongly and positively correlated with DOC concentrations or water colour in natural aquatic systems (Bloom et al., 1991; O'Driscoll et al., 2005).

Although all Hg species can partition into algae (Harris and Ramelow, 1990; Gadd, 1990), only the neutral complexes of MeHg (CH_3HgOH^o and CH_3HgCl^o) have been shown to bioconcentrate in algal tissues and biomagnify through trophic transfer to lethal levels in top organisms of the food chain (Mason et al., 1996; Harris et al., 2003). CH_3HgCl is less polar than CH_3HgOH and is therefore taken up faster by algae (Major et al., 1991). Physical, chemical and biological interactions of uncharged MeHg complexes with the biological barriers of algae lead to direct uptake and bioconcentration of MeHg inside the biota (Boudou et al. 1991).

1.6 Interactions of MeHg complexes with algae

1.6.1 Uptake

Uptake is the process by which MeHg moves from the surrounding medium, crosses the membrane barrier and is transported into algae. Uptake of MeHg into algae proceeds in three main steps, the first of which is adsorption - the accumulation of MeHg at the boundary between the medium and the aquatic membrane without transport into the cytoplasm. This process involves physical, reversible reactions with components of the cell membrane and cell wall. Secondly, transport of MeHg into the cell membrane involves chemical reaction or complexation reaction with protein, lipid and/or polysaccharide molecules located inside the cell membrane (Price and Morel, 1990; Scott and Palmer, 1998). These two steps, together termed "biosorption", lead to membrane-bound MeHg and are scavenging devices most effective in algae and bacteria because of their high surface-to-volume ratio (Michaels and Flegal, 1990). Biosorption in algae is usually very rapid and can be characterized by the Freundlich and Langmiur isotherms (Newman, 1998). These isotherms describe the functional relationships between the adsorbed molecules and the adsorbate at equilibrium. Cell membrane-bound MeHg contribute to total uptake but are less efficiently assimilated by grazers and are easily eliminated with faeces (Reinfelder and Fisher, 1991; Reinfelder et al., 1998).

1.6.2 Bioconcentration

The third and most important step is the intracellular uptake of MeHg across the cell membrane into the cytoplasm where it is sequestered and bioconcentrated in the tissue of the exposed algae. This step termed bioconcentration is usually slower and non-reversible and is the net increase in concentration of MeHg within the algae resulting from the rate of uptake exceeding the rate of elimination (Newman, 1995; Spacie and Hamelink, 1995). Bioconcentration is of great importance in risk assessment because it helps to predict concentrations of MeHg in biota (Mackay and Fraser, 2000).

Bioconcentration in algae is a dynamic process that involves many interconnected variables. The propensity for a chemical to bioconcentrate is dependent on the properties of the chemical (such as its hydrophobicity and lipophilicity), environmental factors (e.g. DOC concentration, trophic status of the water body, redox potential), biotic factors (e.g. the organism's metabolic activity and physiology) and bioavailability of the chemical (e.g. degree of contamination) (Morrison et al., 1996; Gobas et al., 1999; Lee et al., 2000; Shin and Lam, 2001).

1.6.3 Mechanism of MeHg transport

Various mechanisms have been postulated as being utilized in the transport of MeHg into algae. Brief descriptions of possible mechanisms are outlined below in other to elucidate the processes that control MeHg uptake and bioconcentration in algae.

1.6.3.1 Passive diffusion

Passive diffusion is the transport of lipophilic contaminants through the cell membrane via the lipid route, with a rate proportional to their solubility in lipids (determined by their octanol-water partition coefficient (K_{ow}) and inversely proportional to their molecular size. K_{ow} is a physicochemical property representing a chemical's lipophilicity (lipid solubility), where the properties of n-octanol serve as a suitable surrogate for biotic lipid. Chemicals with log K_{ow} values in the range of 2 to about 6.5 are termed lipophilic. This means that such chemicals are a hundred to a million times more soluble in lipids than in water and would therefore bioconcentrate in lipid tissues (Konasewich et al., 1982; Malins et al., 1982). Passive diffusion is a physical process and requires no energy expenditure by the organism. No carriers or binding sites are involved and the rate of transport is directly proportional to the concentration gradient between the outside and inside of the membrane.

1.6.3.2 Facilitated diffusion

Channel proteins or trans-membrane carrier proteins facilitate the transport of water-soluble contaminants into the cell (Stein, 1990). Carriers are protein molecules that bind contaminants on the hydrophobic face of the bi-lipid membrane, diffuse across the membrane and transfer contaminant to the cell interior (Sunda and Huntsman, 1996; Sunda and Huntsman, 1998b). Like passive diffusion, transport in facilitated diffusion occurs down a concentration gradient and the organism does not expend energy.

Frequently, the rate of transport by this process is faster than in passive absorption (Vymazal, 1995). Both passive absorption and facilitated transport are temperature independent (Garnham et al. 1992).

1.6.3.3 Active transport

Transport of contaminants by this process also utilizes carrier proteins similar to the carriers in facilitated diffusion (Vymazal, 1995). Active transport requires metabolic energy, often provided as ATP by photosynthesis in autotrophic organisms. Uptake occurs against a concentration gradient and concentrates contaminants within the cell much more than passive and facilitated diffusion combined (Vymazal, 1995).

Because carrier proteins are employed in both facilitated diffusion and active transport, both processes may be subject to saturation kinetics such that at low external concentrations, the rate of uptake is proportional to the external concentration of the contaminant while at high external concentration, the rate of uptake is limited by the capacity of the carrier proteins and is therefore independent of the external concentration (Vymazal, 1995).

1.7 Bioconcentration Factor BCF

A useful index in environmental risk assessments for quantifying the bioconcentration of contaminants in algae is the bioconcentration factor (BCF). It is the dimensionless ratio

of the contaminant concentration in the aquatic biota (concentration in suspended particulate material per gram dry weight) to concentration in the surrounding medium per gram of water at equilibrium (Watras et al. 1998; Touart, 1995; Zeeman, 1995). Such values give the false impression that all of the MeHg should be associated with the particulate fraction. While the concept of BCF seems straightforward, a number of important environmental considerations are not obvious. MeHg is associated with either the dissolved or particulate fraction of most natural waters (Daughney et al. 2002). The particulate fraction is composed of algae and other plant and animal organic carbon that are retained on a 0.45 μm filter (Rand et al. 1995). In wetlands, rivers and lakes the MeHg in the particulate fraction is only about 10-20% of the MeHg content (Hill, 2005 M.Sc thesis), leaving about 80% available in the dissolved fraction.

The MeHg BCF reported in the literature varies from (10^3-10^6). It was measured in phytoplankton (really particulates) (Watras and Bloom, 1992; Watras et al. 1998; Cleckner et al. 1999). This indicates that phytoplankton or what is retained on a filter (algae, bacteria and detritus) bioconcentrate ambient MeHg in their tissues up to a million times over aqueous concentrations. The MeHg BCF at higher trophic levels is only about 4-7 times higher (Back and Watras, 1995; Watras et al. 1998). The initial BCF at the base of the food web is clearly the most significant.

1.8 Previous Studies

The need for more knowledge on MeHg bioconcentration processes and dynamics at the phytoplankton level of the aquatic food web was identified in the USEPA (1997a) report. Numerous studies on the subject have been initiated in the last decade. Most however, focused on understanding the mechanisms of uptake and bioconcentration in the lower food web. Examples of such studies include Mason et al. (1996), Watras et al. (1998), Golding et al. (2002), and Moye et al. (2002). Despite these endeavours, mechanistic explanations of the processes involved are still highly debated and a universal mechanism for uptake and bioconcentration of MeHg in the lower food web is yet to be defined. For example, while Mason et al. (1996) suggest that uptake in microseston is governed by the passive uptake of neutrally charged MeHg complexes, Watras et al. (1998), found uptake to be the result of active transport of neutral MeHgOH complexes. Furthermore, Golding et al. (2002) claim that uptake of MeHg in microorganisms likely proceeds by facilitated diffusion. Recently, Moye et al. (2002) proposed that MeHg uptake in phytoplankton was the result of several mechanisms functioning simultaneously.

The other studies quantified the BCF of MeHg at the plankton level in the field. For example, Cleckner et al. (1999) measured BCF in periphyton in the Everglades in the range of $10^4 – 10^5$, yet in another study by the same researchers in Northern Everglades, BCF was measured in the range of $10^3 – 10^4$ (Cleckner et al. 1998). Watras and Bloom (1992) measured BCF for phytoplankton in Little Rock lake to be approximately 10^5, while Watras et al. (1998) measured microseston BCF in Winsconsin lakes to range between 10^5- 10^6.

In these field studies such as Watras et al. (1992) and Cleckner et al. (1999), BCF measurements were done with natural lake water samples from which aggregate plankton samples were collected on 0.8 μm filters. These samples may have included zooplankton but were principally phytoplankton of different species, bacteria and detritus. Use of such bulk samples may obscure small but important differences in the measurement of MeHg uptake by phytoplankton as well as the BCF.

Mason et al. (1995), Mason et al. (1996), Lawson and Mason, (1998) and Miles et al. (2001) using laboratory algal cultures, measured BCF to range from 10^4 to $10^{6.7}$. Of the studies cited above, some of the laboratory studies used MeHg and algal concentrations that are several orders of magnitude higher than environmentally realistic levels that may have resulted in gross over –or underestimation of BCF and uptake parameters. For example, Mason et al. (1996) determined BCF and uptake of MeHg by 5- 10 μg (dw) mL^{-1} of algae using 150 pM MeHg (32 ng Hg L^{-1}). Miles et al. (2001) investigated the uptake kinetics of MeHg by algae but under very short exposure periods. In an uptake experiment with medium concentration of 0.3 – 333 nM MeHg (65 –71900 ng Hg L^{-1}), an exposure period of **only 5 minutes** was allowed in the determination of MeHg uptake rates in phytoplankton biomass levels of 5 μg (ww) mL^{-1}. In another similar experiment by Moye et al. (2002), algae were exposed to MeHg for 24 h, at the end of which time the BCF was determined. MeHg uptake by algae was calculated using Freundlich's Isotherm equation - an empirical formula that assumes a linear pattern of adsorption. The assumption of linear kinetics was not validated with a detailed time course experiment.

Though results of these studies all support the conclusion that most MeHg bioconcentration happens in the lower food web, the BCF ranges are very diverse. Further more, the effect of site-specific environmental variables such as aqueous MeHg concentration, algal biomass and dissolved organic carbon (DOC) concentrations in natural systems that would likely have influenced the results of the field studies were not incorporated in the laboratory experiments. Nonetheless, the BCF's for MeHg as determined by these studies have been used to represent bioconcentration at the base of the aquatic food chain and this information has been transferred to some mercury cycling models such as the Regional Mercury Cycling Model (R-MCM - Tetra tech., 1999).

1.9 Factors that affect uptake and algal concentration of MeHg

The following section gives an overview of the environmental variables identified in the literature that might affect MeHg uptake and bioconcentration in algae and thus control the MeHg BCF.

Often, the potential for an organic contaminant to bioconcentrate is greatest for those that are highly lipophilic, as predicted by log K_{ow}. However, the two species of MeHg in aquatic systems have log K_{ow} less than 2 (1.7 for CH_3HgCl; 0.07 for CH_3HgOH) (Bienvenue et al., 1984; Faust, 1992). Neither species, therefore, can be termed "lipophilic", yet their bioconcentration in phytoplankton is comparable to the bioconcentration of lipophilic contaminants like PCBs (polychlorinated biphenyls), which

have log $K_{ow} > 5$ (Mason et al., 1996). Chemicals with log $K_{ow} < 2$ usually bioconcentrate more in nonlipid tissues because of their solubility in water (Connell, 1997). This suggests that other factors must influence the uptake of MeHg into algal tissues.

Major et al. (1991) is of the view that higher algal concentration of MeHg than is predicted by its lipohilicity is the result of MeHg's strong tendency to form complexes with membrane and intracellular ligands like the sulfhydryl groups of proteins, which act as intracellular sinks (Sunda and Huntsman, 1998). Sulphur derived ligands in biota possess sufficient affinity for MeHg that they efficiently remove ionic methylmercury from soluble hydroxide and chloride complexes (Loux, 1998). Burkett (1974) observed from his experiment with live and dead algal cells that uptake and bioconcentration of MeHg in algae depended on the quantity of protein-containing sulfhydryl groups (R-SH) in the algae. Recent findings by Harris et al. (2003) indicate that intercellular MeHg in fish comprise mainly MeHg – RSH complexes.

An organism's physiological and biochemical characteristics, which are dependent on its age and developmental stage, have been found to significantly influence uptake and bioconcentration of MeHg. For instance, Moye et al. (2002) observed significant differences in the uptake capability of 4-day old algal cells and 30-day old cells. Daughney et al. (2001) found a remarkable difference in the sorption capacity of Cd and Fe(III) by bacterial cells in exponential vs. stationary phase. On the other hand,

Daughney et al. (2002) observed no difference in uptake of Hg (II) between bacterial cells of different growth phases.

Abiotic and biotic environmental variables that may affect uptake of MeHg by algae include: spatial and seasonal changes in aqueous MeHg concentration; spatial and temporal changes in algal biomass of water bodies, which influence MeHg cell quota - amount of MeHg per individual cell (Pickhardt et al. 2002); and concentration of complexing and chelating agents like DOC, which reduces the availability of MeHg in its dissolved form (Spacie and Hamelink, 1995). Other abiotic variables include temperature, light intensity and redox potential (Gobbas et al.1999).

No studies were found that incorporated the interactions of environmental variables such as aqueous MeHg concentration, algal biomass and presence of high and low DOC concentration in the determination of MeHg BCF, algal concentration of MeHg or cell quota of MeHg, and in the estimation of MeHg uptake and desorption rate constants at the phytoplankton level, for use in environmental risk assessments. This study was therefore designed to investigate the kinetics of uptake and bioconcentration of MeHg by algae in controlled laboratory experiments using aqueous MeHg concentration, DOC concentrations, and algal biomass levels representative of those found in natural systems.

2. Methods

2.1 Algal Cultures

The test organism used in this study is *Pseudokirchneriella subcapitata* - a unicellular, crescent-shaped (40 to 60 μm^3), fresh water green alga, known until 1990 as *Selenastrum capricornutum* (Hindak, 1990). This alga has been used extensively as a test organism by the USEPA (USEPA, 1994a) and many other agencies. The choice for this organism as test species is based on its:

a) extensive use in algal bioassays;

b) rapid growth and ease to culture in the laboratory;

c) simple and uniform morphology as single cells that seldom clump together thus facilitating counting by hemacytometer and fluorometer.

Culture of algae strain UTCC 66 was obtained from the University of Toronto Culture Collection (UTCC). The stock culture was prepared by aseptically transferring 1 mL of the liquid culture from UTCC into 100 mL of sterilized algal culture medium (AAP – Algal Assay Procedure medium (Environment Canada, 1992) in an autoclaved Erlenmeyer flask and covered with a sterilized cotton stopper to allow the exchange of carbon dioxide and oxygen between the medium and the outside air. The stock culture was maintained at room temperature (24 ± 2^oC) under "cool-white" continuous fluorescent illumination levels of 60 – 80 $\mu E/m^2 \cdot s$. Cultures were placed on a shaker at 100 rpm (revolutions per minute) to ensure that all cells were equally exposed to both light and nutrients and to prevent sedimentation of the algal cells.

Appendix 1 gives the composition of AAP growth medium as adapted from the Environment Canada protocol for growth inhibition test (1992). The pH of the growth medium was adjusted to 7.35 to 7.50 by the addition of 0.1N NaOH and /or 1N $HN0_3$ as recommended in the Environment Canada protocol for growth inhibition test (1992). This pH range allows optimal cellular processes in the test organism and prevents the formation of inorganic precipitates of iron and phosphate (Probert and Klass, 1999).

2.1.1 Batch cultures

Algal growth in batch culture is characterized by four main phases, namely: lag, exponential, stationary and declining. In the lag phase, cells are metabolically active, adapting to the medium, but not growing. In the exponential phase, cell density increases logarithmically as a function of time. In the stationary phase, growth and death rates are in balance. In the declining phase, the cell death exceeds growth because of nutrient depletion, overcrowding and accumulation of toxic by- products.

In order to grow cells in batch-culture, 2 mL of algal stock culture was axenically transferred and cultured weekly in 100 mL of fresh AAP growth medium in autoclaved 250 mL Erlenmeyer flask under continuous illumination and shaking to provide a continuous supply of healthy cells for the study. Each week, 15 mL of algal stock culture was axenically inoculated in 1.5 L of sterilized AAP growth medium in
2.8 L autoclaved Fernbach culture flasks and cultured under continuous light and shaking until 5 days post inoculation when it is harvested for use in uptake experiments with

exponential phase cells. For uptake experiments with stationary phase cells, cells were harvested after 7 days following inoculation.

2.1.2 Semi-continuous cultures

Cell properties such as size, internal nutrient content and metabolic functions can, however, vary considerably among cells in the same growth phase of a batch culture (Kubitschek, 1970). Furthermore, cells in natural environments rarely reach stationary phase before they are ingested (Pick, pers. comm.). In the second part of this study, therefore, cells were grown in a semi-continuous culture. Here, they were maintained in exponential phase by regular addition of nutrients as fresh culture medium and removal of an equal volume of old spent culture medium with the cells contained in it. Chen (1994) observed that cells grown using this method are never without nutrients and their growth conditions resemble those in the natural environment, where there is a continuous new supply of nutrients at low concentrations. When the dilution rate and the growth rate are in balance, the cell density remains constant (Kubistschek, 1970) and experiments can be conducted over long periods of time without the problems of growing new cultures weekly as is associated with batch cultures.

Semi-continuous cultured cells were grown similarly to the batch cultured cells except that since cells were required in the exponential phase, cells were not harvested on the 5th day. Instead, at a specific time from the 5th day, 500 mL of algal culture was axenically withdrawn from the stock culture and replaced axenically by the same quantity of fresh

sterilized AAP growth medium of ambient temperature at a dilution rate of 33% per day. This procedure of standardized withdrawal and replacement was repeated at the same specific time for a period of 4 days. At each time, the 500 mL algal culture removed was measured for cell density.

Cell density was estimated by measuring light transmittance at 750 nm using a Cary 100 BIO UV-Visible spectrophotometer. The greater the cell density, the less light is transmitted through the culture at this wavelength (Spijkerman et al. 1996). Pseudo-steady state was therefore assumed to have been attained when the measured light transmission at 750 nm varied by less than 5% over a period of 4 days, which is when the growth rate became equal to the dilution rate at 33% growth of algae per day.

Algal cultures were kept axenic (devoid of other microbes) by regularly plating algal stock culture on solid nutrient agar during cell harvesting and incubating at 37.5°C for 48 h. If bacterial growth was observed, algal cultures were discarded. No significant bacterial growth was evident. It is recognized that not all strains of bacteria grow on nutrient agar, but no additional tests were deemed necessary.

2.2 Harvesting of algal cells

Algal cells from the batch cultures and semi-continuous cultures were harvested at the desired physiological state or phase by centrifuging at 2000 rpm for 15 min in a Beckman J2-MC centrifuge using a JA-20 rotor. The pellet formed was then washed and re-suspended in a known volume of fresh modified growth medium (MGM) to form the

parent stock (PS) of approximate biomass 0.06 mg (dw) L^{-1}. The MGM was made according to the growth medium protocol by Environment Canada for growth inhibition test but without ethylene diamine tetraacetic acid (EDTA) and micronutrient (see appendix II). Typically, EDTA is used in the preparation of artificial growth medium as a chelator. EDTA as a ligand forms complexes with the metals used in growth medium and decreases the proportion of metals available for uptake by test organisms at a time (Laan et al., 2004). Likewise, chelating substances reduce uptake not only of free metal ions, but also of lipophilic neutral complexes (Boullemant et al., 2004) such as MeHg. For these reasons, EDTA and the other micronutrient were eliminated in the MGM. Appendix 2 gives the chemical composition and concentrations in the MGM. The volume of MGM used for re-suspension depended on the number of experimental units needed.

Initial experiments were conducted using cells grown in batch cultures. Uptake of MeHg by algae was calculated as the difference between total MeHg concentration in the sample and the MeHg concentration of the filtrate as a function of time up to 2 d. In the first experiment and in stationary phase uptake experiments, incubation was for 1 d only. The concentration of MeHg in algae at the end of each interval was the amount of MeHg removed from solution during incubation, divided by the mass of algae in the suspension. The BCF was then calculated as the ratio of the MeHg concentration in the algae ng MeHg g^{-1} algae (ng g^{-1}) divided by MeHg concentration remaining in the growth medium (ng g^{-1} MGM) at the end of each incubation period. Uptake, uptake rate constants, algal concentration of MeHg and BCF were calculated for experiments conducted at a range of MeHg concentration and algal biomass as well as using high- and

low- DOC natural waters. The DOC experiments carried out with batch-cultured organisms were not repeated with semi-continuous cultures. Details of experimental conditions and calculations are described below.

2.3 Algal biomass

In order to obtain the desired biomass of algae for each experiment, the number of cells in the PS was first determined by counting with a hemacytometer. The PS was then diluted with 1L of filter sterilized MGM in 1L high-density polyethylene (HDPE) bottles to give algal biomasses of 0.03, 0.15, 0.3 and 3 mg (dw) L^{-1} for use in all experiments except the DOC experiments. The same algal biomasses were prepared with sterilized natural water samples for the DOC experiments. These cell densities reflect the range of algal biomass found in natural algal habitats.

Table 1 shows the estimated cell densities (cells mL^{-1}), biomass (mg algae L^{-1}) (dw), and the natural habitat represented by the different algal suspensions.

HDPE bottles were pre-cleaned by rinsing three times with de-ionized water, and a mixture of 125 ml of concentrated HCl and 2.5 ml of 2% mixed brominating reagent (made up of equal volumes of potassium bromate and potassium bromide solution) was left to stand in the bottle for 1 week to eliminate possible contamination of the test suspension. Prior to use, 500 μl of 12% hydroxylamine hydrochloride was added to the

brominating mixture to remove all bromine and the bottles were rinsed three times with de-ionized water.

2.4 Uptake experiments

2.4.1 Incubation of cells in artificial medium

Uptake experiments with batch-culture cells in artificial medium were conducted with algal cells both in exponential growth phase and stationary phase. Cells in stationary phase were used in the experiments to compare uptake in exponential vs. stationary growth phases. Uptake experiments carried out in the second part of the study used test organisms grown in semi-continuous cultures to ensure that all cells were in the same physiological state and growing under the same environmental conditions.

In both the batch- and semi-continuous culture experiments, algal suspensions were incubated in growth medium containing known amounts of MeHg for specified time intervals, after which algal cells were removed by filtration and MeHg was measured in the filtrate (and in the filtered algae for semi-continuous culture experiments). From these MeHg measurements, uptake, uptake rate constant, algal concentration of MeHg and BCF were calculated, (see section 4.3).

Triplicate, 1 L suspensions were used for each incubation period in all experiments. The incubation intervals were t = near 0, 0.2, 1, 2, 24 and 48 h. (Incubation at t = near 0 was

really <0.5 minutes because filtration time often depended on the level of algal biomass in the suspension). MeHg concentrations in MGM were adjusted to the desired final concentrations by addition of MeHgCl spike, adapted from Cai et al. (1997).

In the batch culture experiments, the algal biomass concentration ranged from 0.03 to 3 mg (dw) L^{-1}, and aqueous MeHg concentrations ranged from 0.21 to 1.0 ng Hg L^{-1}. The semi-continuous culture experiments extended these ranges to include 0.15 mg (dw) L^{-1} algal biomass, to reflect moderate algal concentrations typical of some natural systems, and 20 ng Hg L^{-1} to reflect contaminated water bodies.

Once spiked, algal suspensions were transferred to an orbital shaker set at 100 rpm to equilibrate in the dark. They were covered with aluminium foil to block out light and to prevent growth. Chlorophyll-a fluorescence was measured every 24 h during the 48 hr total incubation period using a Molecular Device Corporation SPECTRAmax GEMINI XS dual-scanning microplate spectroflourometer. The excitation and emission wavelengths used were 580 nm and 680 nm. No growth dilution was observed.

The experiments also included triplicate 1 L bottles containing: a) spiked MGM without algae; b) MGM blanks containing no MeHg or algae; and c) algal blanks that consisted of algal suspension in MGM without MeHg. The purpose of the spiked MGM (a) was to measure adsorption of MeHg to the filter. The blanks (b) were intended to ensure that the chemicals used for the MGM did not contain significant amounts of MeHg. The algal

blanks (c) were included in order to verify that algae used in the experiment had no MeHg before the experiment.

Each suspension was vacuum-filtered using Whatman GF/C filters with a nominal pore size of 1.2 μm. These filters were found to have low (< 8%) MeHg adsorbtion. Mixed cellulose ester (MCE) filters (0.45 μm) were also evaluated but 30% of MeHg spike was retained on the filter (Table 2). Consequently, Whatman GF/C filters were therefore used throughout the experiments.

Filtrates were preserved with 5 ml of 12 N HCL and stored at 4°C prior to MeHg analysis (Cai et al. 1997). Algal cells collected on the filters were either used for the desorption test (see below) or collected in 40 mL I- Chem vials and stored in the freezer prior to digestion.

2.4.2 MeHg analysis of Filtrate

Analysis of MeHg in the filtrate was done by solid-phase extraction on sulfhydryl cotton fiber columns (SCF) pre-treated to increase sulfide-binding sites. MeHg was isolated as MeHg bromide with acidic-potassium bromide/copper sulphate (2:1) eluent and then extracted into dichloromethane as described by Cai et al. (1997). MeHg concentration in dichloromethane was determined by capillary gas chromatography-atomic fluorescence spectrometry (GC-AFS) and detected, quantified by PS Analytical mercury speciation system model PSA 10.723 with a detection limit of 0.02 ng L^{-1}.

2.4.3 Desorption test

Desorption quantifies the fraction of MeHg adsorbed to the surface of algal cells. At pH 3, all substrate bound MeHg or total Hg is liberated from its substrate (Cai et al., 1997). In this desorption test, algal cells collected on filters were re-suspended in 500 mL of unspiked MGM adjusted to pH 3 and left to equilibrate for 2 h on the shaker after which MeHg was measured in the filtered MGM. The 2 h equilibration period was chosen because in the first uptake experiments, initial rapid uptake was observed in the first 2 h.

2.4.4 Algal digestion

Algae collected on filters were digested in 5 mL of 6 N potassium hydroxide (KOH) and shaken continuously for 4 h to rupture cell walls. After 4 h, pH was adjusted to 3 with 3.2 mL of 6 N HCl to liberate MeHg. 5 mL of acidic-potassium bromide/copper sulphate (3:1) was then added to convert MeHg to MeHgBr. 5 mL of dichloromethane DCM was added to extract MeHgBr from the digestate into the organic phase and the mixture was again left to shake overnight for (12 h). 2mL of DCM was transferred to 1mL of sodium thiosulfate in 7 mL vial to separate MeHg from the debris of digestion. It was shaken for 45 minutes and centrifuged for 15 minutes. 500 μL of sodium thiosulfate was taken out of the 1mL into microcentrifuge vials where 0.3 mL of acidic-potassium bromide/copper sulphate (3:1) and 0.3 mL of DCM was added. It was shaken for 15 minutes and later centrifuged for 2 minutes. Out of the 0.3 mL DCM added, about 0.2 mL was extracted into GC vials as described by Cai et al. (1997) and MeHg quantified using GC-AFS.

Three vials containing 5 ml of 6 N KOH spiked with 300 μl of 5 ng mL^{-1} MeHg were treated similarly in order to measure recovery. Average % recovery of MeHg was 98% ± 0.4% (n =10).

2.4.5 Uptake experiments in high- and low-DOC natural waters

Batch culture algal cells were resuspened in high- and low-DOC natural water samples from the St. Lawrence River (low DOC - 2.8 mg L^{-1}) and Raisin River (high DOC 26 mg L^{-1}). The water samples were collected in 22 L HDPE carboys, previously cleaned like the 1 L HDPE bottles described above. Samples were first filtered through a Nitex 100-micron in-line filter to remove all large particulates including zooplankton and large phytoplankton. In a second filtration step, tangential ultrafiltration using a PALL Tangential Flow Centramate™ Ultrafiltrator with 0.2 μm filter cartridge was used to remove all bacteria and algae. DOC concentrations of the two filtered river water samples were determined using an IO Analytical 1010 total organic carbon analyzer (persulfate oxidation method) followed by IR detection. Water samples were acidified and its inorganic carbon removed.

The first 1 L of the filtrate was discarded to avoid contamination by cartridge storing agent. Subsequent filtrate was collected, inoculated with the appropriate volume of PS, spiked with MeHgCl (as described above) placed on the shaker and sampled at time intervals as described above. Algal suspensions were filtered using Whatman GF/C filters and the filtrate treated similarly as in the experiment above. Water samples from

the St. Lawrence River were spiked with 0.05 mL of 5 ng Hg mL^{-1} of MeHgCl to yield a final concentration of 0.53 ng MeHg L^{-1}. In SLR water samples, the background MeHg concentration was only 0.03 ng Hg L^{-1}. In Raisin River samples the ambient MeHg concentration was 0.21 ng Hg L^{-1} and no additional MeHg was necessary.

2.5 Calculations

2.5.1 Calculation of MeHg uptake by algae

In the batch-culture uptake experiments,

$U_{alg} = MeHg_{spk} - MeHg_{fil}$ (ng Hg L^{-1})

where U_{alg} is the uptake of MeHg by algae (ng Hg L^{-1}), $MeHg_{spk}$ is the MeHg concentration in spiked MGM (ng Hg L^{-1}), and $MeHg_{fil}$ is the MeHg concentration in the filtrate (ng Hg L^{-1}) after a specific incubation time.

In semi-continuous culture uptake experiments, the same calculation was made but in addition, the direct uptake by algae was also measured directly on filters after digestion of algae-

$U_{alg} = MeHg_{dig} / vol_{sus}$ (ng Hg L^{-1})

where $MeHg_{dig}$ (ng) is the mean mass of MeHg measured from digested algal cells (ng) and vol_{sus} (L) is the volume of the algal suspension.

2.5.2 Estimation of MeHg uptake rate constant

In both the batch and semi continuous-culture uptake experiments, the uptake rate constants,

$K_u = \Delta \log U_{alg} / \Delta E_t \, (\mathrm{h}^{-1})$,

where k_u is the uptake rate constant (h^{-1}) and E_t is the exposure . For the rapid and gradual uptake rate constants,

$k_u = \Delta U_{alg} / \Delta E_t \, (\mathrm{h}^{-1})$

2.5.3 Calculation of algal concentration of MeHg

In the batch culture uptake experiments,

$MeHg_{alg} = U_{alg} / M_{alg}$ (ng g^{-1} dry wt. of algae),

where $MeHg_{alg}$ is the algal concentration of MeHg (ng g^{-1}), and M_{alg} is the mass of algae in the suspension (g dry wt.).

Likewise, in the semi-continuous culture uptake experiments,

$MeHg_{alg} = MeHg_{dig} / M_{alg}$

2.5.4 Calculation of algal dry weight

The dry weight of algae in PS (M_{alg}) was determined by drying 15 mL of PS at 60°C for 24 h. The dry weight of same volume of MGM without algal cells was measured in the same manner and the difference between the two weights was taken as the dry weight of algae.

2.5.5 Determination of MeHg BCF in algae

The equilibrium method was used in the determination of BCF.

In the equilibrium method,

$$BCF = C_a / C_m$$ at equilibrium (though there was no equilibrium),

where C_a is algal concentration of MeHg (ng/g algae) and C_m is MeHg concentration in the medium (ng/g media per mL).

2.5.6 Statistical analysis

Significant differences in effects of the treatments (MeHg concentrations, algal biomass and DOC) on uptake and bioconcentration and occurrence of interactions between treatments were determined by 2-way factorial analysis of variance (ANOVA). Linear regression analysis was employed to examine the kinetic relationships of the variables. Data for uptake rates were fit to a logarithmic model using Sigma plot.

3. Results

3.1 Time course of MeHg uptake

3.1.1 Uptake experiment using batch cultures

A suspension containing 0.3 mg (dw) L^{-1} algal biomass (see Table 1) was spiked to a final concentration of 72 ng MeHg L^{-1} and the uptake kinetics monitored for 24 h. Results of this experiment showed the pattern of change in MeHg concentration with time in the filtrate. There was an initial rapid decrease in concentration to 34 ng Hg L^{-1} at the rate of 0.32 ng Hg L^{-1} m^{-1} within the first 2 h followed by a gradual decrease to 16 ng Hg L^{-1} at a rate of 0.01 ng Hg L^{-1} m^{-1} in the remaining 22 h (Fig. 1). No values were obtained between 2 and 24 h and points were joined and not fitted.

Quality control test showed good recovery of MeHg in spiked MGM (92%) after analysis, with less than 9% of MeHg in the spiked medium adsorbed to the bottle walls and GF/C filters (Table 2). The test also showed that MeHg in MGM and algal cells before spiking were below the instrument's detection limit (Table 2).

With the quality control results obtained, change in filtrate concentration with time was therefore taken to be due to uptake of MeHg by algae. Uptake was therefore calculated as the difference in MeHg concentration between the spiked MGM at all data points and the filtrate.

Replotting the above data (Fig. 1) to illustrate uptake by algae, showed that about 50% of the total uptake happened within the first 2 h (Figure 2). Uptake of almost 6% was observed at t = near 0 followed by a further increase to 32% at 0.2 h and 46% at 1 h. After 1 h, uptake started to slow such that only a 6% increase was observed between 1 and 2 h. After 2 h, uptake of MeHg by algae continued to progress gradually to 24 h (see Fig. 2). Total uptake observed at 24 h was 77%.

Figure 2b shows that uptake of 72 ng L^{-1} MeHg may not have stopped at 24 h and would have continued if the exposure time had been extended. The MeHg concentration and algal biomass used in this experiment were higher than the range found in natural waters. Therefore the time course and uptake pattern obtained required further investigation using more environmentally realistic concentrations of MeHg and algal biomass with the exposure time extended to 48 h to provide more data points.

3.1.1.1 Effect of growth phase on MeHg uptake

The above experiment was repeated using 72 ng L^{-1} MeHg and different biomasses of batch culture algae in MGM, as shown (Table1). Uptake of MeHg by batch culture algal cells in exponential and stationary growth phases showed a similar pattern (Fig.3) with more uptake in stationary cells than in exponential cells. In both cases, uptake was rapid in the first 2 h of exposure then continued slowly throughout the rest of the experimental period. In the algal suspension containing exponential cells, 36% (25.7 ng Hg L^{-1}) of the 44% (32.2 ng Hg L^{-1}) uptake of MeHg at 24 h took place in the first 2 h in 0.03 mg L^{-1}

biomass at a rate of 0.21 ng Hg L^{-1} m^{-1}. 38% (26.5 ng Hg L^{-1}) of 63% (44.8 ng Hg L^{-1}) and 43% (31.3 ng Hg L^{-1}) of 79% (57.2 ng Hg L^{-1}) MeHg uptake in 24 h by 0.3 and 3 mg L^{-1} algal biomasses took place in the first 2 h.

With stationary cells, 54% (38.1 ng Hg L^{-1}) of the 79% (56.2 ng Hg L^{-1}) uptake of MeHg at 24 h took place in the first 2 h in 0.03 mg L^{-1} biomass at a rate of 0.31ng Hg L^{-1} m^{-1}. About 56% (39.6 ng Hg L^{-1}) of 82% (57.9 ng Hg L^{-1}) and 74% (52.6 ng Hg L^{-1}) of 98% (71.1 ng Hg L^{-1}) MeHg uptake in 24 h by 0.3 and 3 mg L^{-1} algal biomasses also took place in the first 2 h.

For the same MeHg exposure concentrations, uptake in stationary phase cells was consistently higher than uptake in exponential cells for all three algal biomasses tested. The mean total uptake in stationary cells was higher by 23% ± 7% in 0.03 mg L^{-1} biomass, 18% ± 4% in 0.3 mg L^{-1} algal biomass and 26% ± 6% in 3 mg L^{-1} algal biomass (Table 3).

3.1.1.2 Effect of MeHg concentration on MeHg uptake in artificial medium

Suspension of different biomasses of batch culture algae in 0.5 ng L^{-1} MeHg MGM to reflect environmentally realistic concentrations as shown (Table 1), produced similar change in filtrate concentration over time as was observed in the first 2 experiments with higher MeHg concentrations. There was a rapid initial drop in concentration within the first 2 h followed by slow gradual decrease over the remainder of the 48 h incubation period. However, the magnitude of both the initial and the gradual change in

concentration increased with algal biomass (Fig.4). These values were used to calculate algal uptake (Fig.5). Soon after zero time, uptake was 6% (0.03 ng Hg L^{-1}) in the lowest algal biomass 0.03 mg L^{-1}, 10% (0.05 ng Hg L^{-1}) in 0.3 mg L^{-1} and 14% (0.07 ng Hg L^{-1}) in 3 mg L^{-1}. Uptake at 2 h was 34% (0.17 ng Hg L^{-1}) in 0.03 mg L^{-1}, 42% (0.21 ng Hg L^{-1}) in 0.3 mg L^{-1} and 50% (0.25 ng Hg L^{-1}) in 3 mg L^{-1}. At 24 h, uptake was highest in 3 mg L^{-1} and 0.3 mg L^{-1} algal biomass with total uptake of 78% (0.39 ng Hg L^{-1}) in both, while uptake in 0.03 mg L^{-1} algal biomass was 48% (0.20 ng Hg L^{-1}). At 48 h, uptake increased further in all suspensions, with total uptake of 90% (0.45 ng Hg L^{-1}) in 3 mg L^{-1}, 88% (0.44 ng Hg L^{-1}) in 0.3 mg L^{-1} and 52% (0.26 ng Hg L^{-1}) in 0.03 mg L^{-1} algal biomass. As in the previous experiments most of the uptake took place within the first 2 h of incubation.

Figure 5b shows that uptake did not reach equilibrium in any of the suspensions, regardless of biomass. This was especially evident in the suspension containing the lowest algal biomass. Uptake continued even to the end of the 48 hr incubation period in all the suspensions but with the uptake in the suspensions containing 0.3 mg L^{-1} and 3 mg L^{-1} algal biomass more than the uptake in the suspension with 0.03 mg L^{-1} algal biomass (Fig. 5b).

When the MeHg concentration in the MGM was increased from 0.5 ng Hg L^{-1} to 1.0 ng Hg L^{-1}, the change in filtrate concentration over time followed the same pattern as seen previously. The higher the algal biomass, the greater the observed changes in concentration with time (Fig. 6). Uptake of MeHg in both concentrations (Fig. 5 and

Fig. 7) was low in low-biomass suspension and seemed to be far from reaching equilibrium while uptake in the highest algal biomass almost exhausted the filtrate MeHg by the end of the 48 h incubation period.

In the low-biomass algal suspension (0.03 mg L^{-1}), 30% (0.3 ng Hg L^{-1}) of the uptake in the suspension initially containing 1 ng L^{-1} MeHg took place within the first 2 h. The remaining 24% (0.24 ng Hg L^{-1}) took place between 2 and 48 h. In the suspension containing 0.3 mg L^{-1} algal biomass, 42% (0.42 ng Hg L^{-1}) of the uptake occurred in the first 2 h and 48% (0.48 ng Hg L^{-1}) occurred between 2 and 48 h. In 3 mg L^{-1} algal biomass, 43% (0.43 ng Hg L^{-1}) of the total uptake occurred in the first 2 h and 51% (0.51 ng Hg L^{-1}) occurred in the remaining 46 h.

The aqueous MeHg concentration (0.5 ng L^{-1} and 1 ng L^{-1}) did not have an effect on the total percent uptake of MeHg in same biomass levels of algal suspensions but the absolute uptake was much higher with higher MeHg exposure concentration. (Table 4).

Different slopes were derived when the plot of 0.5 ng L^{-1} and 1 ng L^{-1} MeHg percent uptake vs. exposure time was fit to a logarithmic model. Uptake rate constants estimated from these slopes showed that the rate of MeHg uptake in algae was relative to the aqueous MeHg concentration in the MGM algal suspension. For example, the uptake rate constant of MeHg in MGM algal suspensions initially containing 1 ng L^{-1} MeHg was approximately double that in 0.5 ng L^{-1} MeHg for all three levels of algal biomass tested (Table 5).

3.1.1.3 Effect of algal biomass on MeHg uptake in artificial medium

Both total percent and absolute uptake of MeHg increased with algal biomass. However, increase in uptake with biomass displayed an asymptotic pattern with a greater increase in uptake observed at lower biomass than at higher biomasses. For example, with a 10-fold increase in algal biomass from 0.03 and 0.3 mg L^{-1}, uptake increased by an average of 30% in the algal suspensions of different media. By contrast, only an average 3% increase in uptake was observed with a subsequent 10-fold increase in biomass from 0.3 to 3 mg L^{-1} (Table 4).

3.1.1.4 Effect of high- and low-DOC in natural waters on MeHg uptake

Uptake of MeHg in algal suspensions of St. Lawrence River (SLR) water with low DOC concentration (2.8 mg L^{-1}) and Raisin River (RR) water with high DOC concentration (26 mg L^{-1}) followed the same trend observed in the artificial MGM samples described above: fast initial uptake followed by gradual uptake without reaching equilibrium (Fig. 8 and 9).

In SLR algal suspensions, 41% (0.22 ng Hg L^{-1}) of the 57% (0.30 ng Hg L^{-1}) uptake of MeHg in 0.03 mg mL^{-1} biomass took place in the first 2 h. In the 0.3 and 3 mg L^{-1} biomass suspensions, 49% (0.26 ng Hg L^{-1}) of the 84% (0.45 ng Hg L^{-1}) and 60% (0.32 ng Hg L^{-1}) of the 94% (0.50 ng Hg L^{-1}) uptake, respectively, took place in the first 2 h. In

the RR algal suspensions, 52% (0.11 ng Hg L^{-1}) of the 62% (0.13 ng Hg L^{-1}) uptake in 0.03 mg L^{-1} biomass occurred in the first 2 h. 57% (0.12 ng Hg L^{-1}

Hg L^{-1}) uptake in 0.3 mg L^{-1} and 48% (0.10 ng Hg L^{-1}) of the 67% (0.14 ng Hg L^{-1}) uptake in 3 mg L^{-1} also occurred in the first 2 h. Once again, most of the uptake took place within the first 2 h.

Percent uptake of MeHg in SLR at the end of the 48 h incubation period was very similar in all three suspensions to percent uptake of MeHg in spiked MGM without DOC (Table 4). Uptake of MeHg in RR (26 mg L^{-1} DOC) was, however, different from uptake in both SLR and MGM. Unlike in SLR and MGM, where uptake increased with algal biomass, percent uptake of MeHg in RR at the end of the 48 h incubation period was similar for all suspensions in RR samples, regardless of biomass; 62% in 0.03 mg L^{-1} and 67% in both 0.3 and 3 mg L^{-1} algal biomasses. In the RR samples, ~ 50% of the uptake in all biomasses occurred in the first 2 h, and only about 10 –20% occurred between 2 and 48 h. Uptake after 2 h was gradual and diminishing in all suspensions. In spite of the greater algal surface area in the 0.3 and 3 mg L^{-1} biomass suspensions, uptake after 2 h continued and represented only 0.03 ng MeHg L^{-1} at most (Table 4).

The uptake rate constant of MeHg in RR was different from that estimated for SLR and MGM. The uptake rate constant of MeHg in RR suspensions was almost the same in all the biomasses. This pattern (similar uptake rate constant in all biomass suspensions) is contrary to that observed in SLR and MGM suspensions where the low-biomass suspension always had the lowest uptake rate constant. However, the rate constant in RR

suspensions was related to the aqueous MeHg concentration (Table 5). This shows that despite the unavailability of MeHg in the medium due to binding with DOC, algae was still able to uptake more than 50% of the total MeHg content in the medium especially in the first 2 h of exposure.

3.1.2 Semi-continuous culture (pseudo-steady state) uptake experiments

Experiments using algal cells grown in semi-continuous cultures were conducted to determine if the uptake of MeHg by algal cells measured directly in the algal cells was equivalent to that calculated from loss of MeHg from the media. (Fig. 10).

Increased overall uptake was measured with increased algal biomass. For example, measured uptake over 48 hr in a suspension initially containing 0.5 ng L^{-1} MeHg increased from 48% (0.24 ng Hg L^{-1}) in 0.03 mg L^{-1} algal biomass to 64% (0.32 ng Hg L^{-1}) in 0.15 mg L^{-1} algal biomass. Uptake increased further to 78% (0.39 ng Hg L^{-1}) in 0.3 mg L^{-1} algal biomass and finally to 82% (0.41 ng Hg L^{-1}) in 3 mg L^{-1} algal biomass.

Measured MeHg uptake over 48 h in a suspension initially containing 1 ng L^{-1} MeHg increased from 47% (0.47 ng Hg L^{-1}) in 0.03 mg L^{-1} algal biomass to 62% (0.62 ng Hg L^{-1}) in 0.15 mg L^{-1} algal biomass, further to 68% (0.68 ng Hg L^{-1}) in 0.3 mg L^{-1} algal biomass and finally to 93% (0.93 ng Hg L^{-1}) in 3 mg L^{-1} algal biomass.

In the 20 ng L^{-1} MeHg suspension, measured uptake in 48 hr increased from 55% (11 ng Hg L^{-1}) in 0.03 mg L^{-1} algal biomass to 58% (11.57 ng Hg L^{-1}) in 0.15 mg L^{-1}, 78% (15.5 ng Hg L^{-1}) in 0.3 mg L^{-1} and 94% (18.8 ng Hg L^{-1}) in 3 mg L^{-1}.

In all experiments, most of the uptake took place within the first 2 h of incubation. The digestion protocol however, affected the mass balance such that the measured uptake was lower than the calculated, especially between near zero time and 1h when MeHg uptake was small and not measurable by the method. However, as more MeHg accumulated with time, MeHg in algal cells became measurable. For example, in suspensions containing 20 ng L^{-1} MeHg, 50% (10 ng Hg L^{-1}) of the 67% (13.4 ng Hg L^{-1}) uptake in 0.03 mg L^{-1} algal biomass, 51% (10.2 ng Hg L^{-1}) of the 67% (13.3 ng Hg L^{-1}) in 0.15 mg L^{-1} algal biomass, 52% (10.3 ng Hg L^{-1}) of the 82% (16.4 ng Hg L^{-1}) in 0.3 mg L^{-1} algal biomass and 68% (13.5 ng Hg L^{-1}) of the 96% (19.2 ng Hg L^{-1}) in 3 mg L^{-1} algal biomass was calculated to have taken place in the first 2 h of the 48 h incubation period. But 27% (5.4 ng Hg L^{-1}) out of 50%, 21% (4.17 ng Hg L^{-1}) out of 51%, 45% (9 ng Hg L^{-1}) of the 52% and 55% (11 ng Hg L^{-1}) of the 68% uptake in 2 h was measured in 0.03,0.15, 0.3 and 3 mg L^{-1} algal biomasses respectively.

In suspensions containing 1 ng L^{-1} MeHg, 34% (0.34 ng Hg L^{-1}) of the 48% (0.48 ng Hg L^{-1}) total uptake in 0.03 mg L^{-1} algal biomass, 39% (0.39 ng Hg L^{-1}) of the 63% (63 ng Hg L^{-1}) in 0.15 mg L^{-1} algal biomass, 42% (0.42 ng Hg L^{-1}) of the 73% (0.73 ng Hg L^{-1}) in 0.3 mg L^{-1} algal biomass and 58% (0.58 ng Hg L^{-1}) of the 93%(0.95 ng Hg L^{-1}) in 3 mg L^{-1} algal biomass took place in the first 2 h of exposure. But 15% (0.15 ng Hg L^{-1}) of

34%, 32% (0.32 ng Hg L^{-1}) of the 39%, 32% (0.32 ng Hg L^{-1}) of the 42% and (50% (0.50 ng Hg L^{-1}) of the 58% uptake in the first 2 h of exposure was measured in 0.03,0.15, 0.3 and 3 mg L^{-1} algal biomasses respectively.

In suspensions containing 0.5 ng L^{-1} MeHg, 42% (0.21 ng Hg L^{-1}) of 58% (0.29 ng Hg L^{-1}) total uptake in 0.03 mg L^{-1} algal biomass, 58% (0.29 ng Hg L^{-1}) of the 70% (0.35 ng Hg L^{-1}) in 0.15 mg L^{-1} algal biomass, 64% (0.32 ng Hg L^{-1}) of the 78% (0.39 ng Hg L^{-1}) in 0.3 mg L^{-1} algal biomass and 82% (0.41 ng Hg L^{-1}) of the 92% (0.46 ng Hg L^{-1}) in 3 mg L^{-1} algal biomass took place in the first 2 h of exposure. However, 20% (0.1 ng Hg L^{-1}) of the 42%, 40% (0.2 ng Hg L^{-1}) of the 58%, 40% (0.2 ng Hg L^{-1}) of the 64% and 36% (0.18 ng Hg L^{-1}) of the 82% uptake in the first 2 h of exposure was measured in 0.03,0.15, 0.3 and 3 mg L^{-1} algal biomasses respectively. The greatest difference occurred at low biomass with few exceptions e.g. (Fig. 12 sect. 1D).

3.2 Desorption test

Desorption of MeHg from algal cells was similar in the two MeHg concentrations tested (0.5 ng Hg L^{-1} and 1 ng Hg L^{-1}). Percent of MeHg desorbed was higher in MeHg taken up in the first 2 h than MeHg taken up subsequently between 2 and 48 hrs (Fig. 11). Average desorbed MeHg was < 10% of total uptake at 48 h in both aqueous MeHg concentrations.

For example, in experiments with suspensions initially containing 0.5 ng Hg L^{-1} MeHg, an average of 40% ± 3% of the mean MeHg taken up (0.12 ± 0.01 ng Hg L^{-1}) by all

biomasses at 0.2 h was washable. An average of 31% ±8% of the 0.16 ± 0.01 ng Hg L^{-1} MeHg taken up after 1 h, 22% ± 7% of the 0.21 ± 0.02 ng Hg L^{-1} taken up after 2 h, 13% ± 4% of the 0.33 ± 0.06 ng Hg L^{-1} MeHg taken up after 24 h and 12% ± 3% of the 0.38 ± 0.06 ng Hg L^{-1} MeHg taken up after 48 h were washable within the 2h equilibration period of the desorpion test.

In experiments initially containing 1 ng L^{-1} MeHg, an average of 17% ± 2% of 0.22 ± 0.01 ng L^{-1} MeHg taken up after 0.2 h in all biomasses was washable, 21% ± 6% of the 0.31 ± 0.02 ng L^{-1} MeHg taken up after 1h, 18% ± 5% of 0.38 ± 0.04 ng L^{-1} MeHg taken up after 2 h, 18% ± 5% of 0.69 ± 0.13 ng L^{-1} MeHg taken up after 24 h and 6% ± 3% of 0.79 ± 0.13 ng L^{-1} MeHg taken up after 48 h were washable within the 2 h equilibration period. The average concentration of MeHg desorbed from 0.5 and 1 ng L^{-1} MeHg concentrations within the 2 h equilibration of the desorption test was 0.05 ±0.02 ng L^{-1}.

3.3 Growth test

Chlorophyll-a fluorescence was measured in order to ensure that growth did not occur during the 48 h exposure period. A slight decrease in chlorophyll-a fluorescence (from 13.3 to 12.3 QSU) was observed, indicating loss in algal fluorescence and confirming that uptake over the time course of the experiment was not increased as a result of growth dilution (Fig.12).

3.4 Effects of environmental variables on algal concentration of MeHg

Algal concentration of MeHg in both the batch culture and semi-continuous culture uptake experiments depended on the aqueous MeHg concentration (Table 6 and 9). A significant positive correlation ($r^2 = 0.976$, $p < 0.001$) was observed between MeHg concentration and algal concentration of MeHg. For example, for both types of cultures, algal concentration of MeHg after incubation in 1 ng Hg L^{-1} aqueous MeHg was twice that of cells incubated in 0.5 ng L^{-1} MeHg.

In general, algal concentration of MeHg decreased with increase in algal biomass. Statistical analysis by linear regression and factorial ANOVA of algal concentration of MeHg vs. biomass confirmed a significant negative correlation ($r^2 = 0.855$, $p < 0.001$) (Table 6).

Algal concentration of MeHg was not affected by the presence of DOC in SLR water, but in high-DOC RR water, a slight reduction in algal concentration of MeHg (average 35%) was observed in suspensions containing 0.3 and 3 mg L^{-1} algal biomass (Table 6). However, since ambient MeHg concentration was used in the uptake experiment, most of the MeHg may already be associated with DOC.

3.5 BCF

BCF calculations for MeHg in algae after 48 h by equilibrium method produced similar results in all algal suspensions of media tested except in RR water sample, where Log

BCF in 0.3 and 3 mg L^{-1} algal biomasses was lower by 0.1 than in suspensions containing the same biomass but different DOC and MeHg concentrations (Tables 6). Statistical analysis using factorial ANOVA of MeHg concentration and BCF demonstrates that BCF of MeHg in algae was independent of aqueous MeHg concentration ($p=0.2$).

A strong negative relationship ($r^2 = 0.994$, $p < 0.0001$) was observed between algal biomass and BCF in all the algal suspensions (Table 6).

Table 1. Characteristics of the algal suspensions in MGM, SLR and RR showing algal biomass (mg (dw) L^{-1}), cell estimate (cells mL^{-1}) and the natural habitat that is simulated.

Algal biomass ($mg\ L^{-1}$) DW	Cell estimate (Cells mL^{-1})	Reflected natural habitat
0.03	4 000	ultraoligotrophic
0.05	20 000	oligotrophic
0.3	40 000	mesotrophic
3.0	400 000	eutrophic

** MGM – Modified growth medium, SLR – St. Lawrence River and RR – Raisin River.

Table 2. Quality control test for recovery, accuracy and precision

Sample	MeHg in medium (ng L^{-1}) before Analysis	Measured MeHg (ng L^{-1}) after Analysis	% Recovery	Sample size (n)
Spiked MGM (GF/C filter)	72	66.2 ± 0.72	92	10
Spiked MGM (MCE filter)	72	51.4 ± 0.44	71	8
MGM blank	Below Detection limit	Below Detection limit	----	6
Algal blank	Below Detection limit	Below Detection limit	----	6

** MGM – Modified growth medium, GF/C filter – Glass Fibre filter, MCE – Mixed cellulose ester filter.

Table 3. Comparison of total MeHg uptake over a 24 hr exposure period, by exponential phase vs. stationary phase algal cells. Mean uptake ± S.E of three replicates are presented.

	Mean Total uptake of MeHg (ng L^{-1})	
Algal biomass (mg (dw) L^{-1})	Exponential phase	Stationary phase
0.03	32 ± 2.0	54 ± 0.6
0.3	45 ± 1.4	58 ± 0.1
3	57 ± 0.4	71 ± 0.01

Table 4. MeHg uptake from aqueous MeHg concentrations ranging from 0.21 to 20 ng MeHg L^{-1} by 0.03, 0.3 and 3 mg (dw) L^{-1} algal biomasses over 48 h exposure. Mean uptake ± S.E of three replicates are presented with total percentage (%) in parenthesis.

		Total MeHg Uptake (ng MeHg L^{-1}) (%)		
		Algal Biomass (mg (dw) L^{-1})		
Medium	MeHg Concentration (ng L^{-1})	0.03	0.3	3
RR (High DOC)	0.21	0.13 ± 0.02 (62%)	0.14 ± 0.01 (67%)	0.15 ± 0.02 (71%)
SLR (Low DOC)	0.53	0.30 ± 0.01 (57%)	0.45 ± 0.01 (90%)	0.50 ± 0.01 (94%)
MGM	0.50	0.26 ± 0.1 (54%)	0.44 ± 0.01 (89%)	0.45 ± 0.01 (90%)
MGM	1.0	0.54 ± 0.07 (54%)	0.90 ± 0.01 (90%)	0.94 ± 0.03 (94%)
MGM	20.0	13.4 ± 0.3 (67%)	16.4 ± 0.4 (82%)	19.2 ± 0.02 (96%)

Table 5. MeHg overall uptake rate constant k_u (h^{-1}) of MeHg over 48 h incubation period in 0.03, 0.3 and 3 mg (dw) L^{-1} algal biomasses in batch suspensions of different aqueous MeHg concentrations. R^2 are presented in parentheses.

		Uptake rate constant (h^{-1}) (R^2)		
		Algal Biomass (mg (dw) L^{-1})		
Medium	MeHg Concentration (ng L^{-1})	0.03	0.3	3
RR (High DOC)	0.21	0.04 (0.62)	0.04 (0.60)	0.03 (0.73)
SLR (Low DOC)	0.53	0.08 (0.83)	0.12 (0.91)	0.14 (0.84)
MGM	0.50	0.07 (0.73)	0.12 (0.94)	0.12 (0.92)
MGM	1.0	0.13(0.78)	0.24(0.94)	0.26(0.95)

Table 6. Algal concentration of MeHg after 48 h incubation period in 0.03, 0.3 and 3 mg (dw) L^{-1} algal biomasses in suspensions of different aqueous MeHg concentration. Mean ± S.E of three replicates are presented.

		Algal Concentration of MeHg (ng g^{-1})		
		Algal Biomass (mg (dw) ml^{-1})		
Medium	MeHg Concentration (ng L^{-1})	0.03	0.3	3
RR (High DOC)	0.21	2111 ± 56.0	228 ± 5.3	24 ± 0.7
SLR (Low DOC)	0.53	4666 ± 96.0	722 ± 20.1	83 ± 0.3
MGM	0.50	4111 ± 200.0	722 ± 5.3	73 ± 2.3
MGM	1.0	9350 ± 1581	1537 ± 27.0	152 ± 2.3

Table 7. Log BCF of MeHg after 48 h in 0.03, 0.3 and 3 mg (dw) L^{-1} algal biomasses in different algal suspensions determined by the equilibrium method. Mean ± S.E of three replicates are presented.

		Mean Log BCF (± S.E)		
		Algal Biomass (mg (dw) L^{-1})		
Medium	MeHg Concentration (ng L^{-1})	0.03	0.3	3
RR (High DOC)	0.21	7.0 ± 0.012	6.0 ± 0.01	5.1 ± 0.01
SLR (Low DOC)	0.53	6.9± 0.009	6.1± 0.012	5.2± 0.003
MGM	0.50	6.9± 0.02	6.2± 0.003	5.2± 0.012
MGM	1.0	6.9± 0.09	6.2± 0.007	5.2± 0.007
MGM	20.0	6.9± 0.3	6.1± 0.4	5.2± 0.02

Table 8. Measured MeHg uptake rate constant (h^{-1}) in 0.03, 0.3 and 3 mg L^{-1} algal biomasses of cells in pseudo steady state conditions over 48 h exposure period. (R^2) in parenthesis.

		Uptake rate (h^{-1}) (R^2)		
		Algal Biomass (mg (dw) L^{-1})		
Medium	MeHg Concentration (ng L^{-1})	0.03	0.3	3
MGM	0.50	0.06 (0.947)	0.09 (0.883)	0.10 (0.936)
MGM	1.0	0.12 (0.982)	0.14 (0.862)	0.20 (0.866)
MGM	20.0	1. 3(0.818)	1.9 (0.908)	3.2 (0.943)

Table 9. Algal concentration of MeHg (ng g^{-1}) in 0.03, 0.15, 0.3 and 3 algal biomasses of cells in pseudo steady state conditions over 48 h exposure period. Mean algal concentration ± S.E of three replicates are presented.

		Algal concentration of MeHg (ng g^{-1})			
		Algal biomass (mg (dw) L^{-1})			
Medium	MeHg Concentration (ng L^{-1})	0.03	0.15	0.3	3
MGM	0.5	3450± 570	1050± 303	525± 204	68± 8.6
MGM	1	7750± 1323	2250± 361	1125± 215	159± 7.6
MGM	20	152000± 30046	32100± 12276	18400± 3174	2600± 333

Figure 1. Filtrate MeHg concentration (ng L^{-1}) over 24 h exposure period of a medium with 3 mg (dw) L-1 algal biomass of exponentially grown algal cells and initially spiked to 72 ng L-1 MeHg.

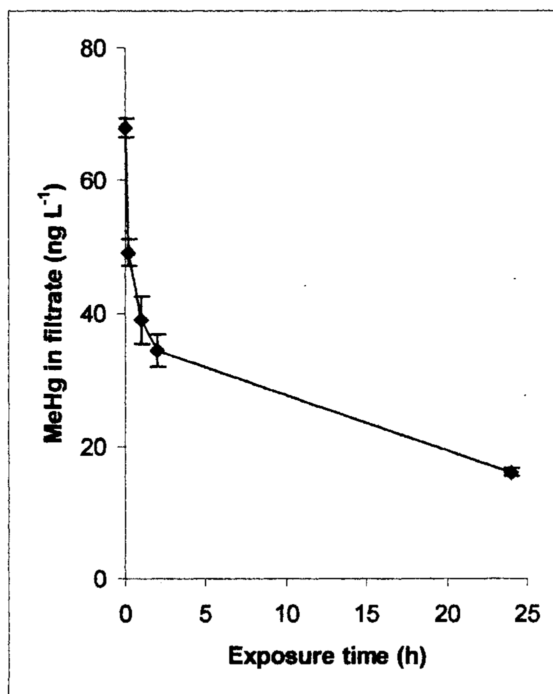
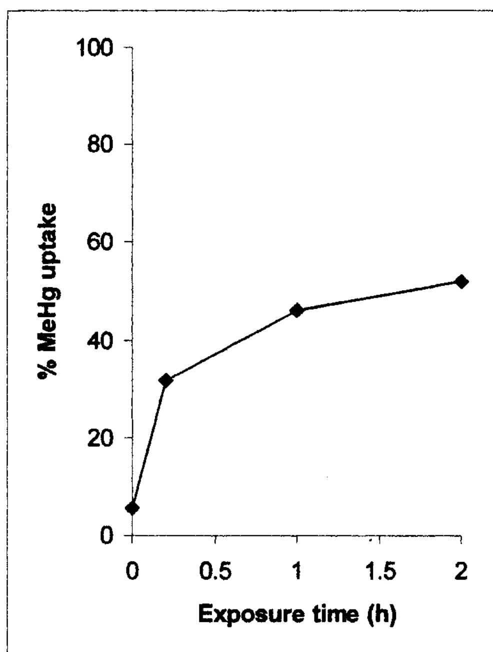
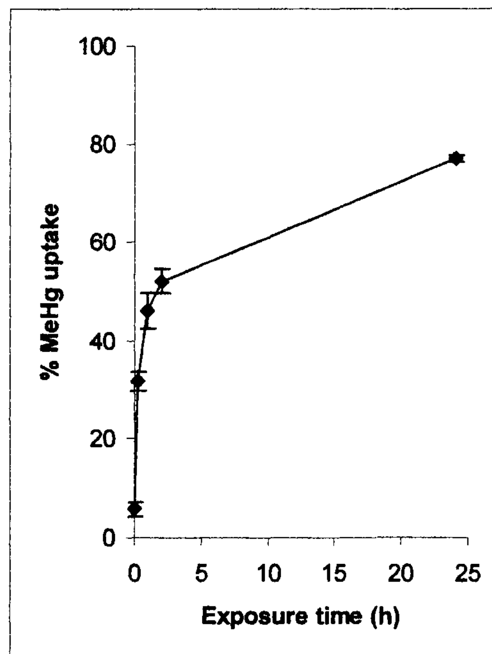

80
60
40
20
0
MeHg in filtrate (ng L-1)
0
5
10
15
20
25
Exposure time (h)

Figure 2. (a) Mean uptake (n=3) of MeHg (72 ng L^{-1}) by algae over the first 2 h of exposure. (b) Mean uptake (n=3) of the same MeHg concentration over 24 h of exposure. Error bars represent ± SE.

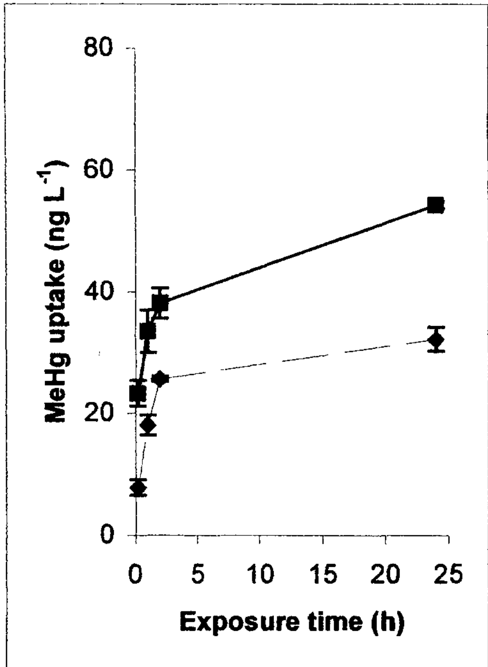


(a)

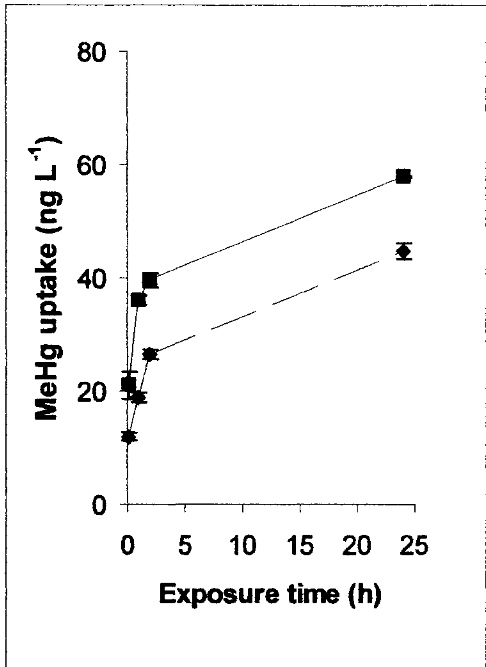


(b)

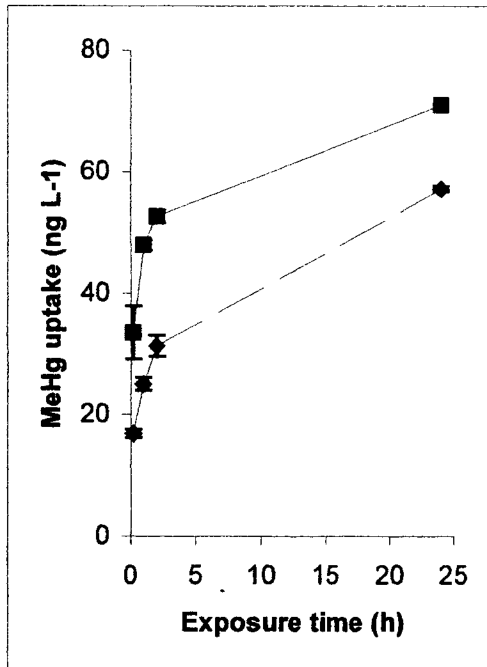
Figure 3. Uptake in 72 ng L^{-1} MeHg suspensions by (a) 0.03, (b) 0.3 and (c) 3 mg (dw) L^{-1} algal biomasses of cells in exponential (diamonds) vs. stationary (squares) growth phases.



(a)

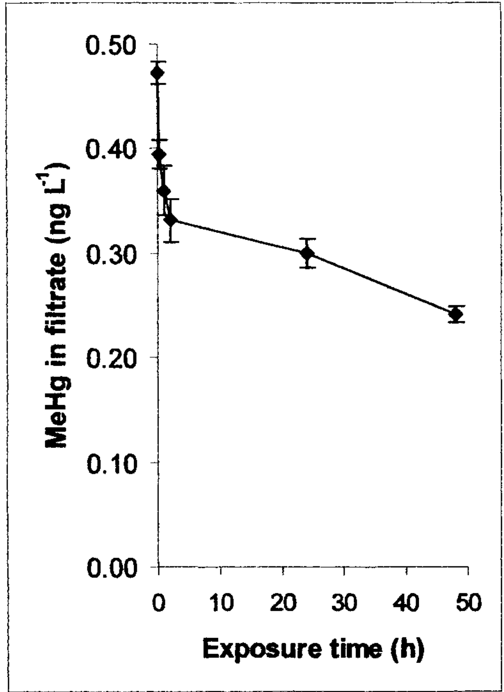


(b)

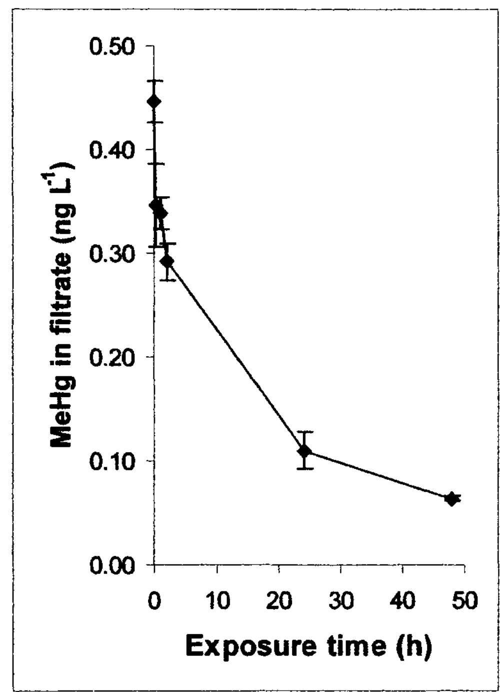


(c)

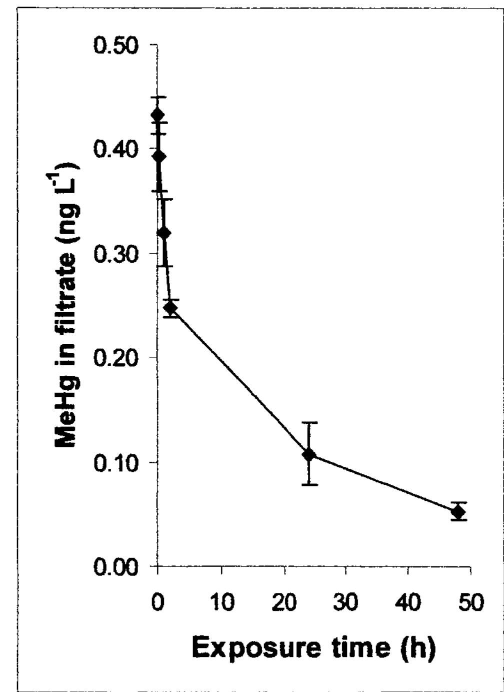
Figure 4. Change in MeHg concentration (± S.E) in (a) 0.03, (b) 0.3 and (c) 3 mg (dw) L^{-1} algal biomass in MGM suspensions initially containing 0.5 ng L^{-1} MeHg over 48 h incubation period.



(a)

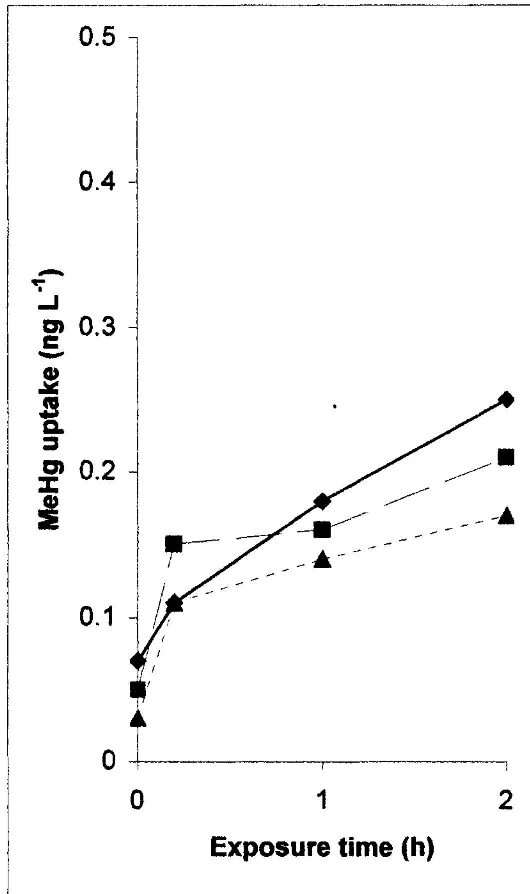


(b)

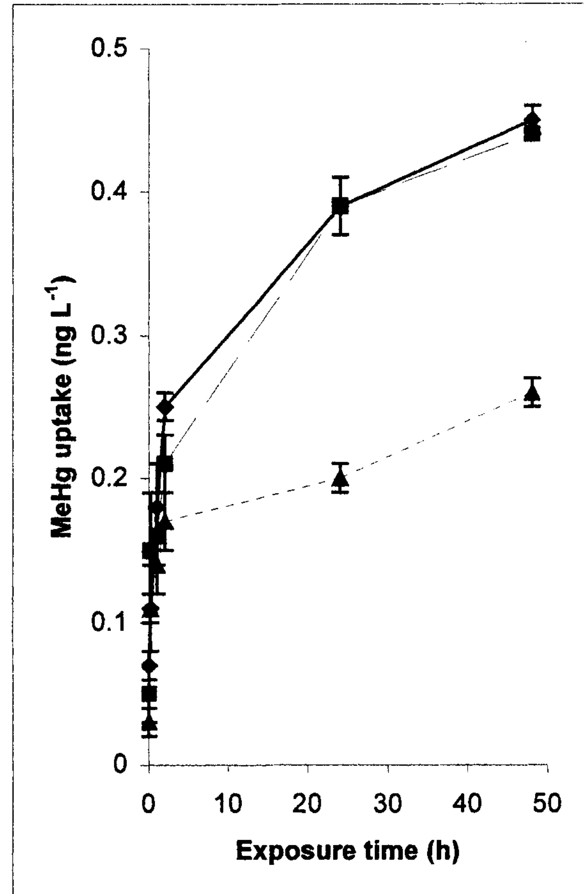


(c)

Figure 5. (a) MeHg uptake in 0.5 ng L^{-1} MeHg MGM suspensions containing 0.03 (triangles), 0.3 (squares) and 3 (diamonds) mg mL^{-1} algal biomass of *Pseudokirchineriella subcapitata* over 2 h exposure. (b) Over 48 h exposure. Mean values ± SE (n=3) is presented.

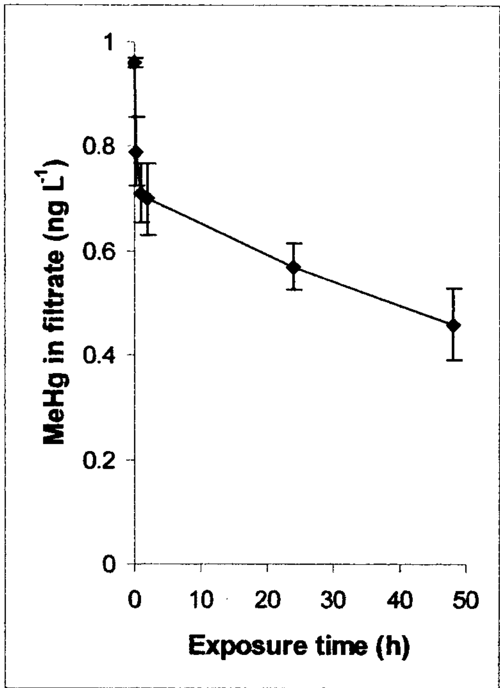


(a)

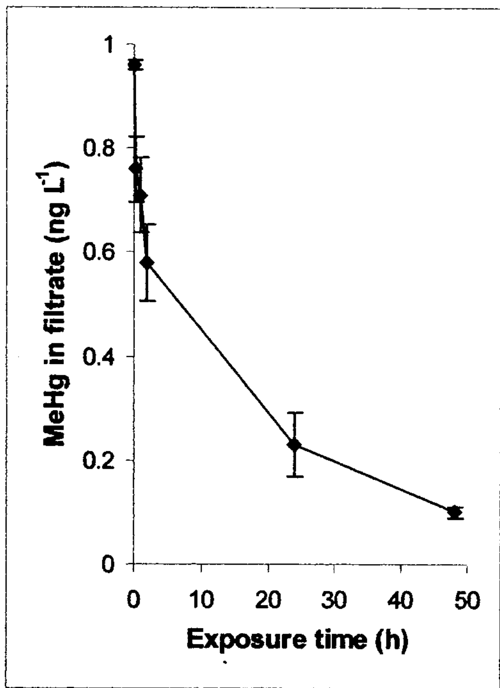


(b)

Figure 6 Change in MeHg concentration in filtrate of suspensions with an initial concentration of 1.0 ng L^{-1} over 48 hr incubation of (a) 0.03, (b) 0.3 and (c) 3 mg (dw) L^{-1} algal biomasses. Error bars represent ± SE (n=3).



(a)



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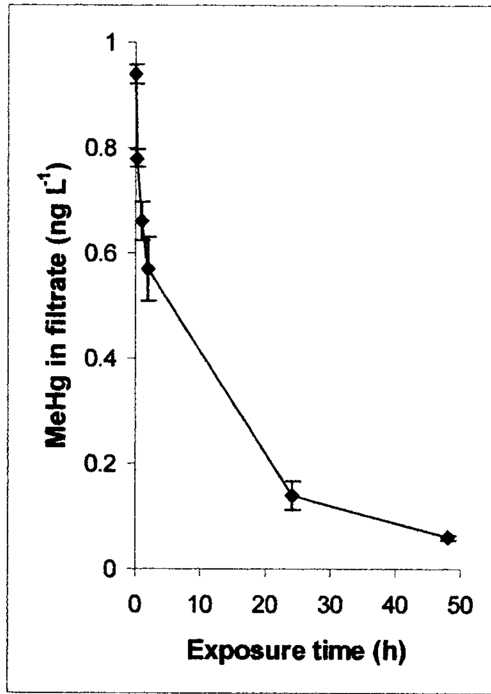

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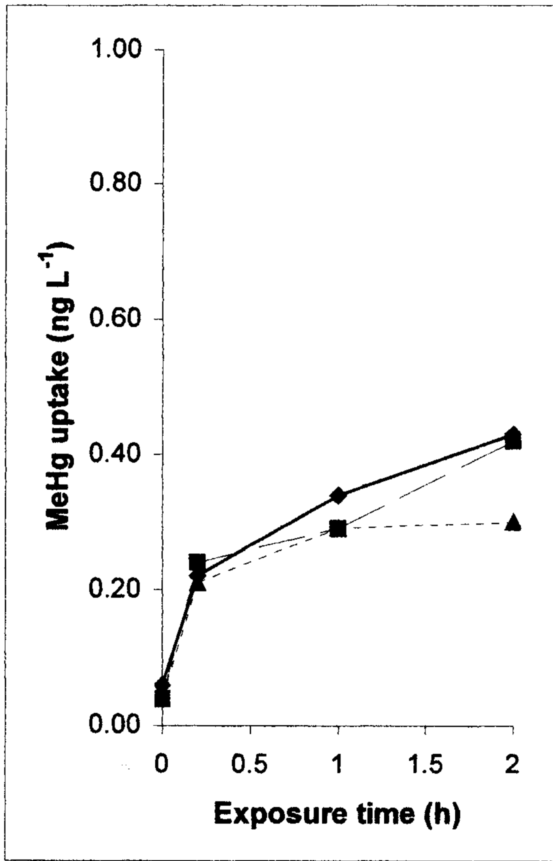
Figure 7. (a) Uptake in 1 ng L^{-1} MeHg suspension by 0.03 (triangles), 0.3 (squares) and 3 (diamonds) mg (dw) L^{-1} algal biomasses of *Pseudokirchineriella subcapitata* over 2 hrs exposure. (b) Same uptake over 48 hrs exposure times. Mean totals (± S.E) of 3 replicates are presented.



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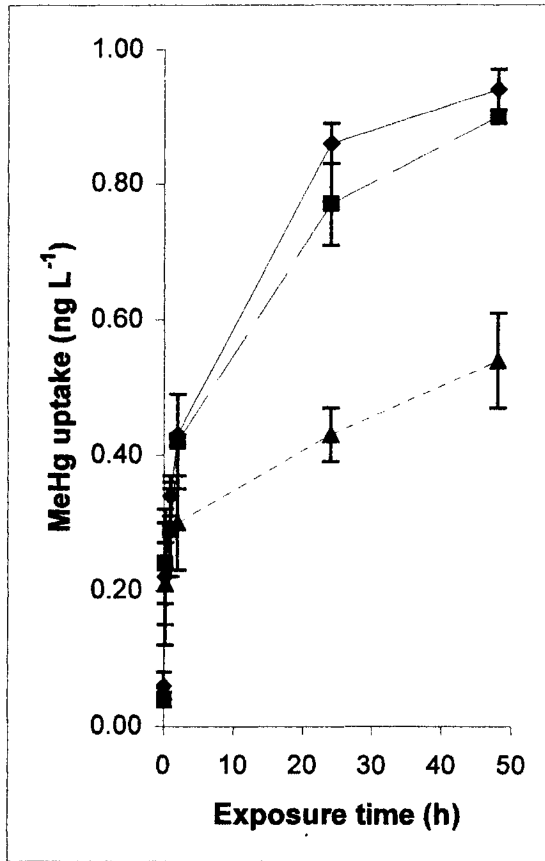
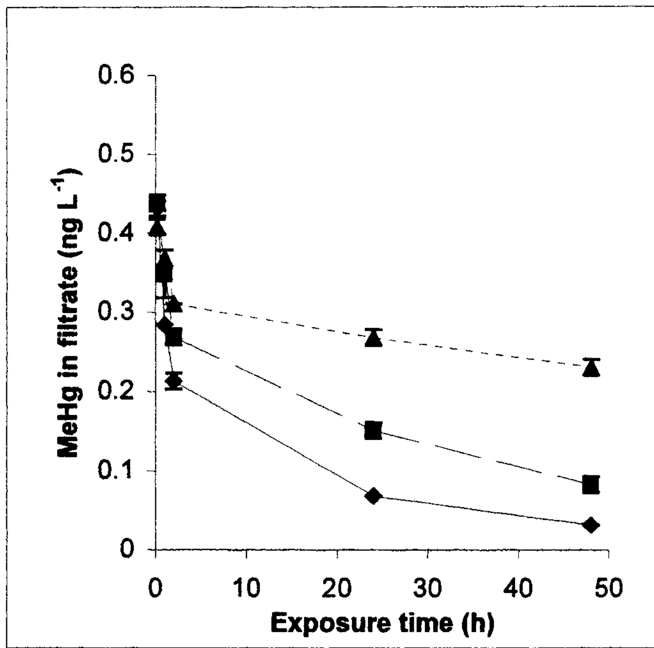
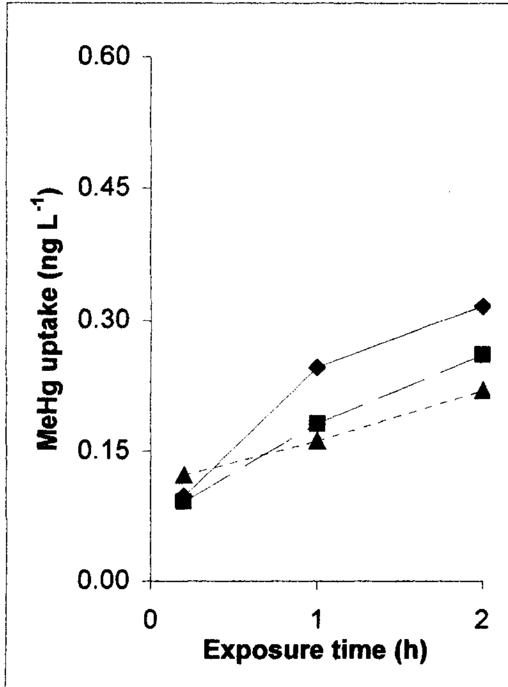


(b)

Figure 8. a) Change in MeHg concentration of St. Lawrence River water sample SLR (0.53 ng Hg L^{-1}) over 48 h of incubation and b) uptake of MeHg by 3 algal biomasses – 0.03 (triangles), 0.3 (squares) and 3 (diamonds) mg L^{-1} over 2 h of exposure. c) Same uptake as above over 48 h. Mean totals (± S.E) presented.



(a)



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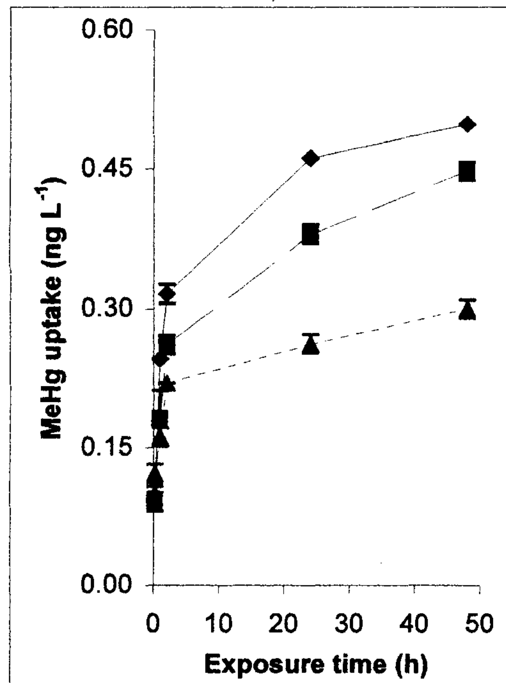
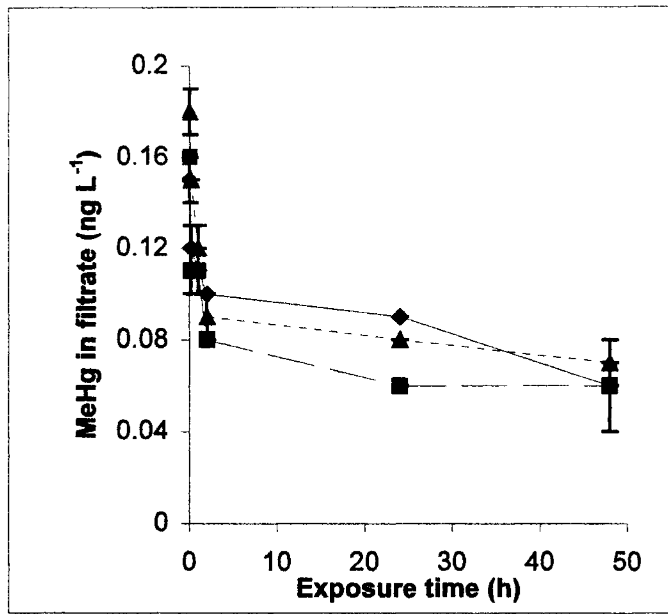
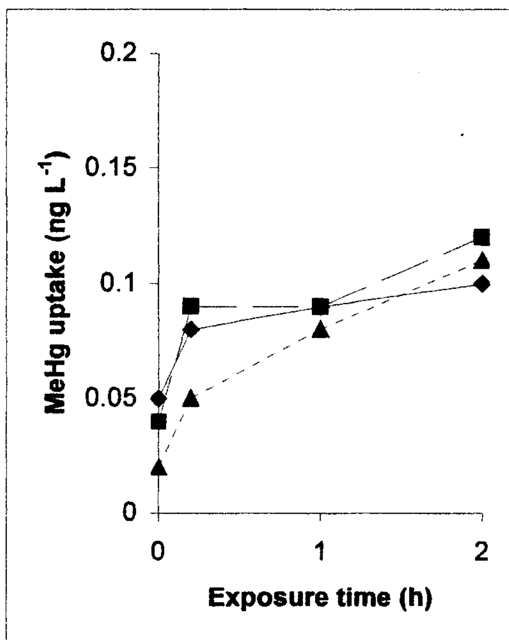


(c)

Figure 9. a) Change in MeHg concentration of Raisin River water sample RR (0.21 ng Hg L^{-1}) over 48 h incubation by 3 algal biomasses – 0.03, 0.3 and 3 mg L^{-1} over 2 h of exposure b) uptake of MeHg in Raisin River suspension c) same uptake over 48 h. Mean totals (± S.E) presented.(n=3).



(a)



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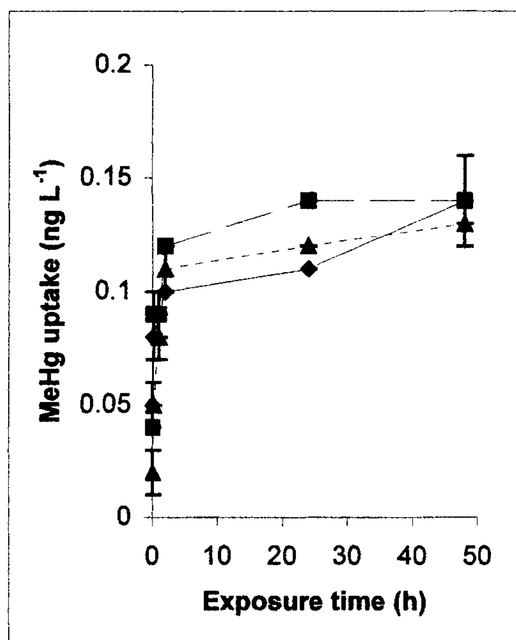
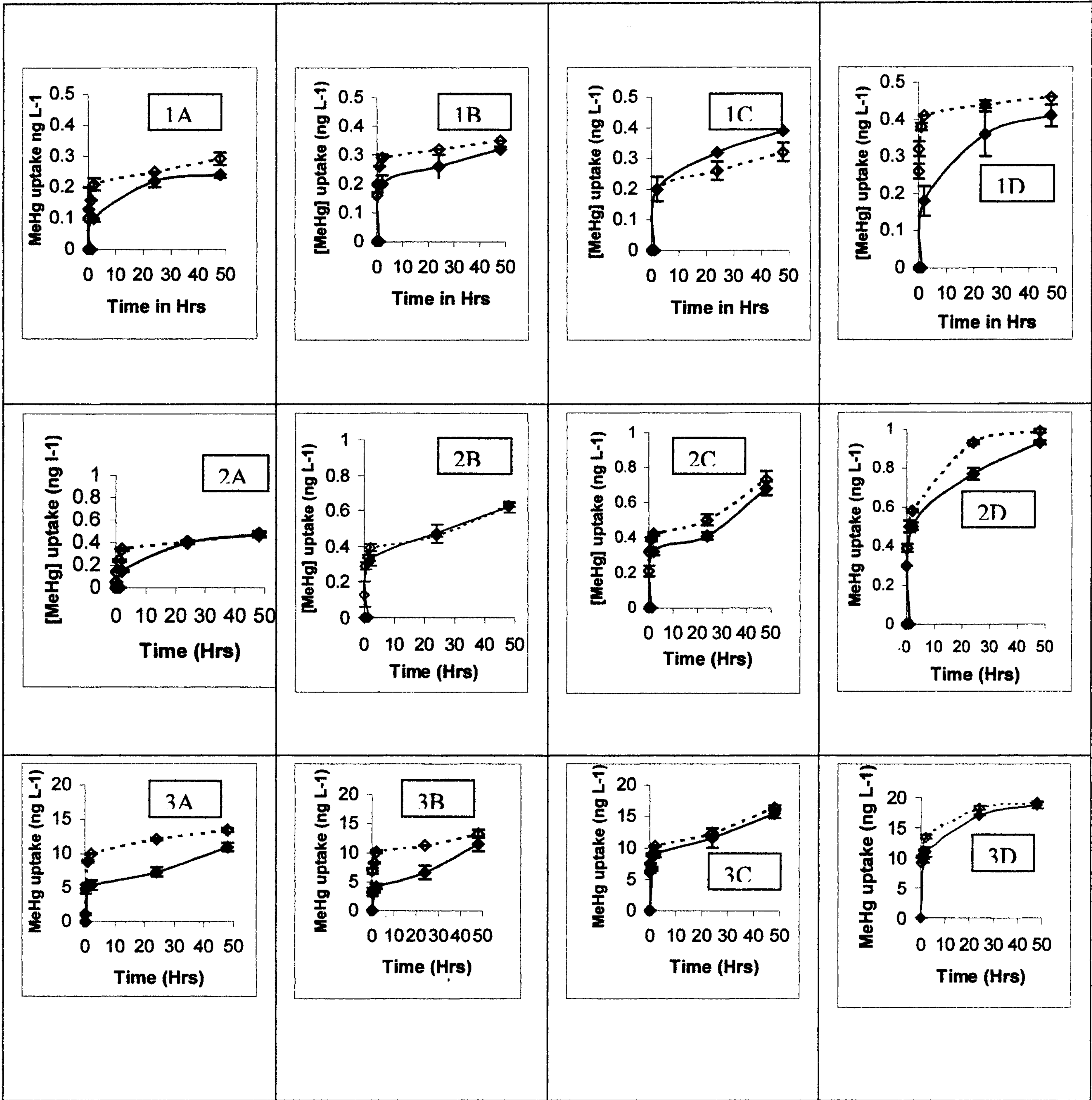


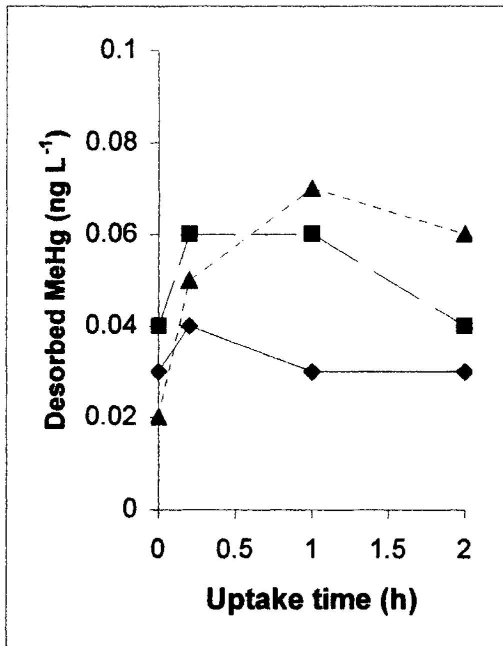
(c)

Figure 10. Measured (solid line) and calculated (dashed line) uptake in 0.5,1.0 and 20.0 ng L^{-1} MeHg shown as 1,2,3, respectively and A, B, C and D represents 0.03,0.15, 0.3 and 3 mg L^{-1} algal biomasses suspended in MGM over 48 h incubation period.

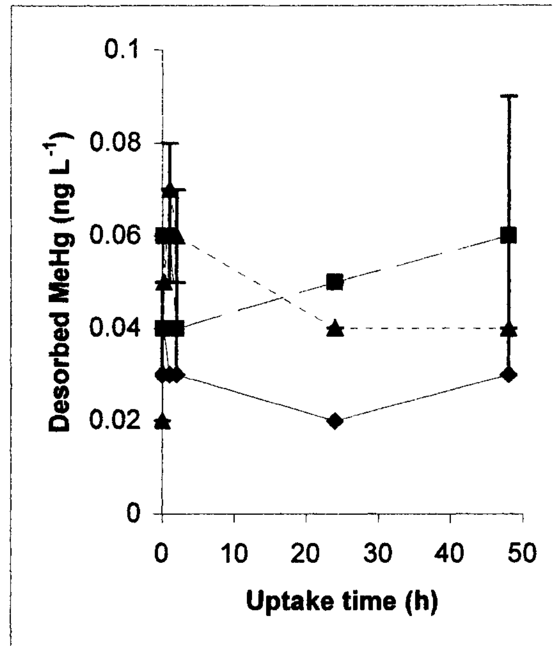


--------- Calculated uptake _______Measured uptake

Figure 11. MeHg Desorption from uptakes at different exposure times in 2 h experimental time at (a) 0.5 and (c) 1.0 ng MeHg L^{-1} and to 48 h for (b) 0.5 and (d) 1.0 ng MeHg L^{-1}.



(a)



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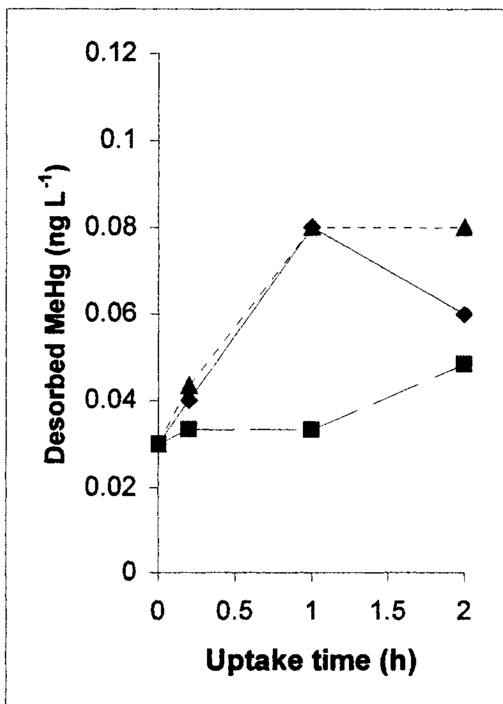

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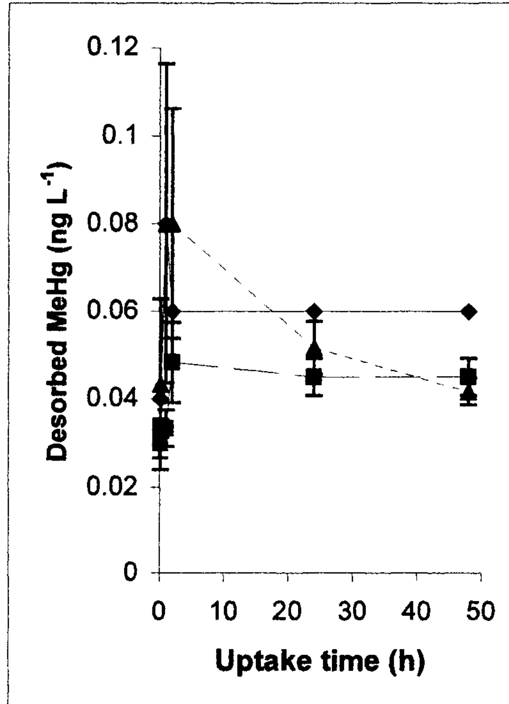
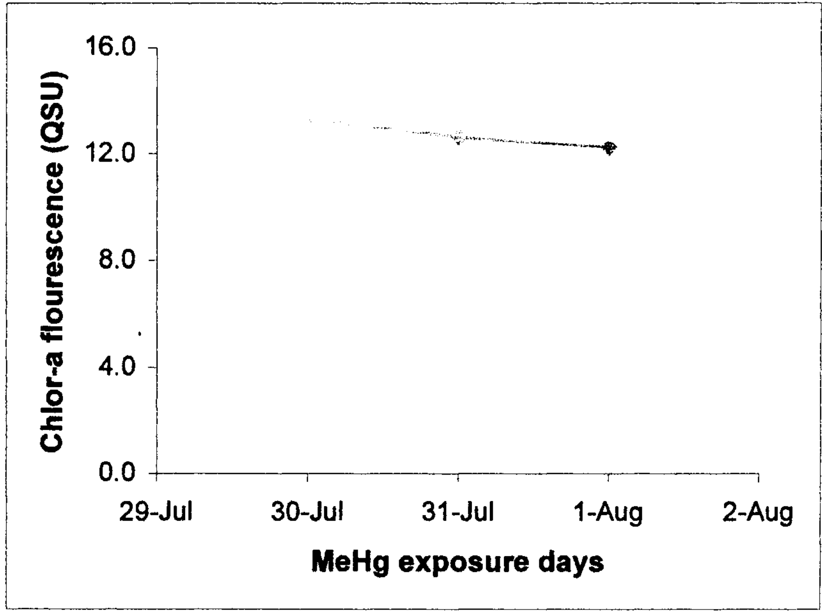


(d)

Figure 12. Chlorophyll-a fluorescence growth checks on algal cells over 48 hr.


16.0
12.0
8.0
4.0
0.0
Chlor-a flourescence (QSU)
29-Jul
30-Jul
31-Jul
1-Aug
2-Aug
MeHg exposure days

4. Discussion and Conclusions

4.1 Time course of MeHg uptake by algae

MeHg uptake pattern by algae was similar in all the uptake experiments in this study. Increasing MeHg concentrations resulted in increased rapid and gradual uptake phases. Higher algal biomasses enhanced the uptake rate of MeHg but decreased the amount per cell or per unit biomass. The time course was characteristically asymptotic with initial rapid uptake in the first 2 h of incubation, followed by gradual uptake for the remainder of the 48 h incubation period. This observation demonstrates the large uptake and bioconcentration capacity of this alga.

The result of this study, showing great capacity of the test organism in uptake and bioconcentration of MeHg, is in agreement with findings of another study on the uptake of PCB. In that study, uptake experiments were carried out using *Chlorella vulgaris*, another green alga of the same family (Chlorophycae). Substantial uptake and bioconcentration capacity was observed in the distribution of PCB between the organism and the test water (Swackhamer and Skoglund, 1993).

The rapid uptake observed in the first two hours is assumed to be scavenging of MeHg by adsorption and absorption processes into the "Donnan free space" on the algal cell wall and cell membrane. Donnan free space is the space immediately next to surfaces of algae and other plant cells (dead or alive), endowed with negatively charged sites where cations

are weakly bound and concentrated to facilitate their transport into cells (Glass, 1988; Marschner, 1995). MeHg in these experiments is likely taken up as the neutrally charged MeHgOH complex (confirmed by MINEQL analysis because of the pH of the medium), but on the biological matrix, ligand exchange occurs and the methyl mercuric ion (CH_3Hg^+) binds readily to sulfhydryl groups in the Donnan free space from where it is transported into the cell interior by either passive, facilitated diffusion or active transport. As CH_3Hg^+ is transported into the cells, more MeHgOH converge onto the cell surface as CH_3Hg^+ is introduced into the free space.

The transport of MeHg into algal cells was very gradual and may have been affected by the interaction of the OH group of MeHg with water molecules in the medium which have been shown to retard the rate at which MeHgOH is taken up into biota in contrast to the uptake of MeHgCl (Faust 1992). However, MeHg uptake continued throughout the course of 48 h incubation period and did not reach plateau or steady state typical of transport-assisted mechanisms (i.e. facilitated diffusion or active transport). Nonetheless, uptake experiments were conducted in the dark, so active transport may have been limited. The absence of saturation phase suggests a passive diffusion as the possible mechanism of uptake under the given experimental conditions and under the range of MeHg concentrations tested.

The time course for uptake depended on the amount of algal biomass in the suspension. Uptake of MeHg in the medium was fastest and almost complete at 48 h in suspensions

with higher algal biomass. In some of the lower biomass suspensions, up to 45% of MeHg was left in the medium at the end of 48 h.

Chlorophyll-a fluorescence tests confirmed the absence of growth during the 48 h incubation period. This result together with the occurrence of about 50% of the total uptake in the first 2 h, i.e. when the cells would have been adjusting to their new environment (lag phase), confirms that uptake of MeHg by algae under the given experimental condition was not influenced by biodilution through algal growth.

Desorption of MeHg from algal cells was generally < 10% of uptake with a higher % desorption within the first 2 h than from MeHg uptake later in the incubation period. The amount desorbed was relatively constant. This observation suggests that in the first 2 h, initial binding of MeHg to the cell surface was weak and therefore could be desorbed. The subsequent, gradual uptake of MeHg, more likely represents sequestration of MeHg within the algal cells by formation of coordination bond with R-SH groups in proteins either inside the membrane as membrane-bound or in the cytoplasm, hence the minimal desorption observed after the initial 2 h period. Like all strong coordination bonds, CH_3Hg-SH displays strong covalent character (Harris, 2003).

4.2 Factors affecting uptake and bioconcentration in algae

In the following sections, each of the environmental variables tested (aqueous MeHg concentration, algal biomass, DOC will be discussed in terms of their impact on: a) total uptake, b) uptake and desorption rates, c) algal concentration of MeHg, and d) BCF.

4.2.1 Aqueous MeHg concentration

4.2.1.1 Percent Uptake

Total percent uptake was similar in the range of aqueous MeHg concentration (0.2 – 20 ng Hg L^{-1}) tested (Table 4). Uptake was almost complete (> 90%) in all cases with high algal biomass, perhaps because of the large surface to volume ratio of *P. subcapitata*. Furthermore, binding of MeHg to R-SH inside the cell violates the assumptions of equilibrium partitioning (Spacie et al. 1995) such that uptake of MeHg was dependent on the quantity of binding sites. More membrane surface provides more unfilled binding sites in the Donnan free space, which facilitate greater uptake.

Since in all the high-biomass suspensions, *P. subcapitata* took up almost all the MeHg in solution irrespective of the concentration, it can be deduced that aqueous MeHg concentration did not influence the percent uptake of MeHg from the suspension. Rather, the availability of cell surfaces and membranes determined the percent uptake at any time. This suggests a diffusion-limited process with an unlimited capacity for uptake. Instead uptake is limited by the MeHg exposure concentration. This phenomenon may be different in natural systems, where the supply of MeHg is constantly replenished.

However, the effect of aqueous MeHg exposure concentration to uptake of MeHg by algae is displayed in the rate of uptake and in the algal concentration of MeHg. As the aqueous MeHg in the medium increased, the rate of uptake of MeHg and the amount of MeHg accumulated inside the cell also increased.

4.2.1.2 Uptake and desorption rate constants.

The overall uptake rate constant K_u increased consistently with increase in aqueous MeHg concentration. For instance, a 40-fold increase in aqueous MeHg concentration in the semi-continuous culture suspensions yielded an average 45-fold increase in the calculated overall uptake rate constants of the 4 biomasses tested. Likewise with a 20-fold increase in MeHg concentration, the calculated overall rate constant increased by an average factor of 25 in all the biomasses (Appendix 5). In the measured uptake rate constant, similar proportional increase was observed (Table 8).

It can therefore be concluded that the uptake rate constant of MeHg in algae increased proportionally with the ambient MeHg concentration.

The overall uptake rate constants described above are the combined rate constants of the 2 phases of uptake -rapid and gradual. However, when the data for the rapid uptake was not included, a linear uptake pattern was observed between t = 2 h and 48 h. The uptake rate constant derived for the gradual uptake of MeHg within this period was on average ~40 times lower than the overall uptake rate constant K_u and rapid uptake rate constant in

both batch and semi-continuous cultures. The rapid uptake rate constant was more comparable to the overall uptake rate constant (Appendix 3,4).

Estimation of MeHg uptake from the gradual uptake rate constant alone would underestimate the uptake of MeHg into algae while the estimation of MeHg uptake can be achieved from the rapid uptake rate constant only.

The average desorbed MeHg measured after acid desorption was 0.05 ng MeHg L^{-1}. Calculation of desorption rate constant was therefore very difficult. However such insignificant desorption of MeHg from algal cells corroborates that MeHg taken up is retained and accumulated within the cell hence the high BCF. This finding further validates algal concentration of MeHg to be as a result of the rate of uptake far exceeding the rate of elimination.

4.2.1.3 Algal concentration of MeHg

A significant positive correlation was observed between algal concentrations of MeHg and the range of aqueous MeHg concentrations tested ($r^2 = 0.98$). This result is consistent with the findings of Kirkwood et al. (1999) in which more MeHg in phytoplankton was measured in the hypolimnion of a dystrophic lake where aqueous MeHg concentration increased over the course of the summer season than in phytoplankton collected from the metalimnion where aqueous MeHg levels remained constant over the same time interval.

4.2.1.4 BCF

BCF after 48 h was similar over the range of aqueous MeHg concentrations tested. Since percent MeHg by the same biomass of algae was comparable across the aqueous MeHg concentrations tested, and furthermore, if the percent of MeHg associated with particulate fraction (algae inclusive) is consistent (Hill, 2005), it is understandable that the BCF (the ratio of MeHg concentration in algae to that in the medium) was independent of the aqueous MeHg concentration in the suspension.

Rationally, time should be the determining factor of BCF because algal concentration of MeHg increased with time. In this study, BCF increased with time and not with MeHg concentration. Based on this result, the null hypothesis that aqueous MeHg concentration will not affect BCF is therefore accepted.

4.2.2 Algal biomass

4.2.2.1 Uptake

Uptake increased with algal biomass. This result is not surprising given that more algal biomass provides more binding sites required for biosorption of MeHg. ANOVA analysis showed algal biomass as statistically significant to uptake ($p < 0.01$), the null hypothesis that algal biomass does not affect uptake of MeHg is therefore rejected. This result is consistent with the results of Kirkwood et al. (1999), in which they observed that

as the mass of phytoplankton increased, the mass of mercury associated with it increased also.

The increase in MeHg uptake with biomass, however, was not proportional to the increase in algal biomass. There seemed to be a biomass threshold for maximum uptake of MeHg after which little or no uptake would occur. For example, MeHg uptake only doubled with a 10- to 100-fold increase in algal biomass. This resulted in much lower algal concentration and individual cell quota of MeHg in suspensions with high algal biomass. The threshold biomass observed in this study is 0.3 mg (dw) L^{-1} – biomass level typical of most mesotrophic systems (Lean, pers. Comm.).

This observation is in agreement with the phenomenon of biodilution as described by Pickhardt et al. (2002), in which the algal concentration and individual cell quota of MeHg was lowered by the distribution of the same amount of MeHg among a greater number of algal cells resulting from growth.

4.2.2.2 MeHg uptake rate constant

Uptake rate constant increased with increase in algal biomass. Here again, proportional increase in the uptake rate constant with algal biomass was limited beyond the threshold biomass.

4.2.2.3 Algal concentration of MeHg

Algal concentration of MeHg was negatively correlated with algal biomass ($r^2 = 0.86$). Though the uptake rate was increased with increase in algal biomass, the presence of more cells in the suspension entailed distribution of MeHg in many compartments. This reduced the MeHg cell quota of individual cells in the suspension and the overall MeHg content of the biomass. This result demonstrates that in most eutrophic systems with high algal biomass, MeHg is transferred less efficiently into the food chain than in oligotrophic systems with lower algal biomass.

4.2.2.4 BCF

A strong negative correlation ($r^2 = 0.99$) was observed between algal biomass and BCF. Consistent with this observation is the measurement of high BCF in the hypolimnion of a lake, which contained a relatively low density of viable algal cells in contrast to the measurement of low BCF in the metalimnion of the same lake, where greater viable algal cell density was observed (Kirkwood et al., 1999).

In this study, BCF values were similar in media of different aqueous MeHg concentration. Variation in MeHg BCF was as a result of the different algal biomasses used. Variation in algal biomass may therefore explain to a great extent the diverse MeHg BCF in phytoplankton reported in previous studies, as shown in the introduction.

4.2.3 DOC

4.2.3.1 Total uptake

It has been hypothesized by other authors that complexation of MeHg to DOC in most aquatic systems reduces its bioavailability in the water column (Hudson et al., 1994; Watras et al., 1995). Uptake of MeHg by algae in the presence of low DOC (2.8 mg L^{-1}) as in the SLR samples was similar to uptake in MGM without DOC. This observation supports the above hypothesis, as it could be explained that in SLR suspension, the low DOC complexed only an insignificant fraction of MeHg out of the solution, thereby most of the MeHg was available for uptake by algae. However, uptake of MeHg in high-DOC (26 mg L^{-1}) RR water samples was slightly different. Total uptake and % uptake was comparable in all the biomasses. In the presence of higher algal biomass, the usual pattern of increased uptake with increase in algal biomass was not observed because most of the MeHg was associated with the high DOC. Instead, a 10- to 100-fold increase in algal biomass resulted in only a 5% marginal increase in percent uptake (from 62% to 67%) over the course of the experiment.

It is possible that in high DOC water sample, even though MeHg present in the sample was the ambient MeHg concentration, there was free, dissolved MeHg (i.e. not bound to DOC), available to fill up the membrane binding sites on the algal surface for onward transport into the cell within the first 2 h. As the time progressed, only trivial amount was available for uptake.

This result is in agreement with studies by Sijm et al. (1995) and Kirkwood et al. (1999), in which they observed reduced uptake of PCBs and MeHg in the presence of high DOC. This result further corroborates the hypotheses in the literature that DOC reduces availability of MeHg for uptake. However, the small sample size (n = 3) and the use of ambient MeHg concentration do not allow a definitive conclusion on this point.

4.2.3.2 Uptake rate constant of MeHg

In the presence of low DOC, DOC had no effect on the uptake rate constant of MeHg. However, in the presence of high DOC, all the uptake rate constants – overall, rapid and gradual were almost similar in all the biomasses. Uptake of MeHg was limited to the first 2 h of uptake, after which non-availability of dissolved MeHg in the suspension, may have led to the little or no uptake of MeHg in the gradual uptake phase (Appendix 3).

4.2.3.3 Concentration of MeHg in algae

Algal concentration of MeHg in suspensions containing high DOC and high algal biomass was reduced by 30% when compared with algal concentration of MeHg in SLR with low-DOC and MGM with no DOC. Algal concentration of MeHg in the low biomass suspension was comparable with algal concentration in the low-biomass of other suspensions. The percentage of MeHg taken up by algae (~ 70%) is greater than the percentage bound to DOC (~ 30%). By using ambient MeHg concentration, only ~30% (0.06 ng MeHg L^{-1}) of the total MeHg was associated with DOC while about ~70% (0.14

ng MeHg L^{-1}) was available for uptake by algae. This result suggests that in the presence of high DOC, MeHg is still bioavailable for uptake by algae.

4.2.3.4 BCF

There was no observed effect of DOC on BCF. The 30% decline in uptake of MeHg observed in the high biomasses resulted in a slight decline (0.1) in the log BCF of MeHg in these suspensions.

4.2.4 Growth phase

4.2.4.1 Total uptake

Uptake of MeHg in algal cells in stationary phase was consistently higher than in exponential cells. This observation supports previous observations made in this study with respect to greater uptake with greater membrane surfaces. Cells in stationary phase are usually larger and have more organelles with more membrane surface area than the younger, smaller cells in exponential phase.

4.2.4.2 Uptake rate of MeHg

The uptake rate of MeHg was higher in stationary phase cells than exponential phase cells. The uptake rate in both exponential and stationary phase cells increased with increase in biomass. Higher uptake of MeHg by cells grown by batch culturing to

stationary phase in contrast to cells grown by the same method to exponential phase necessitated the use of cells grown to exponential phase by semi-continuous culturing in other to better simulate conditions found in natural environments when MeHg uptake is measured directly from digested algal cells.

4.2.4.3 Comparison of uptake and concentration of MeHg in algal cells grown in batch and semi-continuous cultures.

A comparison of MeHg uptake in cells grown to exponential phase by both culturing methods showed that uptake pattern and uptake rate constant of MeHg was similar in cells from both culture types (Appendix 3,4). The only observed difference was in the algal concentration of MeHg.

Algal concentration of MeHg was consistently lower in exponential cells from the semi-continuous cultures than in exponential phase cells grown in batch cultures. This observation is best explained by the conclusions of Kubitscheck (1970) that in batch cultures, cells of different growth phases and therefore of different biochemical composition are usually mixed together. Cells grown to exponential phase in batch cultures, for example, may be mixed with cells in lag, stationary and decline phases. The different biochemical composition of these cells would have enhanced uptake and algal concentration of MeHg. Cells from the semi-continuous, "pseudo steady-state" culture would have had higher metabolic activity but lower biochemical composition and size, hence the lower algal concentration of MeHg.

Overall, calculated and measured MeHg uptake maintained similar pattern of uptake except in places where differences in mass balance existed. The differences were observed more in low biomass and low concentrations especially within the first 2 h of uptake when MeHg accumulated may have been undetectable by the method of digestion used. Within this time also, it is assumed that MeHg is still bound within the membrane. We postulate that there may be a form of Hg present especially at low aqueous MeHg concentration that is not extracted efficiently at this time. Furthermore, the tight covalent bond between MeHg and the carrier molecules especially during the early phase of MeHg uptake may have been underestimated by the digestion analysis of particulate MeHg used.

4.3 Conclusions

In this study, algal biomass was identified as an important parameter in the uptake and bioconcentration of MeHg in algae –affecting the time course of MeHg uptake, % uptake of MeHg, algal concentration of MeHg, cell quota of MeHg and MeHg BCF. The diverse BCF recorded in the literature could be explained by variation in algal biomass of those studies.

This study clearly shows that estimation of MeHg uptake by algae is best achieved by the use of either the rapid or the overall uptake rate constant. Use of the gradual rate constant underestimates the actual uptake.

The presence of DOC has been hypothesized to reduce availability of MeHg for uptake by algae. Results of this study do not contradict this hypothesis, but emphasize that though there is competition between algae and DOC for MeHg in aquatic systems, MeHg is always available for uptake by algae. Algae have scavenging abilities that enhance uptake of substantial amount of MeHg in the presence of DOC.

Indeed the chemistry of SLR was different from MGM but the results were identical with little role for the DOC. For RR no MeHg was added in the experiment and consequently much of what was there was indeed associated with DOC, only the free or bioavailable MeHg were taken up. It is therefore not surprising to see the kinetics in RR samples, different from that seen in SLR and MGM samples.

However, more research in the uptake of MeHg in the presence of different concentrations of DOC would further validate this finding.

The presence of a biomass threshold in MeHg uptake by algae observed in this study should be investigated further. Identification of biomass threshold would be useful in risk assessment strategies.

This study confirms that algae such as the one used here have great capacity to uptake and bioconcentrate contaminants in the environment. Such high uptake capacity could be achieved by the contaminants mimicking the structure and behaviour of compounds vital to the organism.

This study clearly provides details of one of the most critical steps in the uptake of MeHg in the aquatic food chain for further integration into the wise management of this toxic chemical.

The need for the identification of the exact mechanism(s) of MeHg uptake by algae cannot be overemphasized especially in risk assessment.

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Appendices

Appendix 1 Final concentration of macronutrients and micronutrients in AAP growth culture medium.
(Adapted from Environment Canada, 1992)

Macronutrient	Element	Concentration (μg/L)
$NaNO_3$	N	2630
$MgCl_2 . 6H_2O$	Mg	1650
$CaCl_2 . 2H_2O$	Ca	750
$MgSO_4 . 7H_2O$	S	1200
K_2HPO_4	P	120
$NaHCO_3$	Na	6880
	K	293
	C	1340
Micronutrient	**Element**	**Concentration (μg/L)**
H_3BO_3	B	20.27
$MnCl_2 . 4H_2O$	Mn	72.11
$ZnCl_2$	Zn	0.98
$CoCl_2 . 6H_2O$	Co	0.22
$CuCl_2 . 2H_2O$	Cu	0.003
$Na_2MoO_4 . 2H_2O$	Mo	1.8
$FeCl_3 . 6H_2O$	Fe	20.7
$Na_2EDTA . 2H_2O$	--	----

Appendix 2. Nutrient composition of the artificial medium MGM used in MeHg uptake experiments by algae.

Macronutrient	Element	Concentration (μg/L)
$NaNO_3$	N	2630
$MgSO_4 . 7H_2O$	S	1200
K_2HPO_4	P	120
$NaHCO_3$	Na	6880
	K	293
	C	1340

Appendix 3. Uptake rates of MeHg in rapid, gradual and overall uptake by 0.03, 0.3 and 3 mg (dw) L^{-1} algal biomasses of cells grown in a batch culture. R^2 in parenthesis.

Uptake rates (h^{-1})							
MeHg (ng Hg L^{-1}) in Medium	Algal biomass (mg (dw) L^{-1})	Rapid uptake (0-2 h)	(R^2)	Gradual uptake (2-48 h)	(R^2)	Overall uptake (0-48 h)	(R^2)
RR – 0.21	**0.03**	0.03	0.93	0.0004	0.99	0.04	0.88
	0.3	0.03	0.71	0.0004	0.73	0.04	0.84
	3.0	0.02	0.72	0.0009	0.94	0.03	0.89
SLR – 0.53	**0.03**	0.06	0.99	0.002	0.99	0.08	0.83
	0.3	0.09	0.77	0.004	0.97	0.12	0.91
	3.0	0.12	0.93	0.004	0.89	0.14	0.84
MGM – 0.5	**0.03**	0.06	0.76	0.002	0.97	0.07	0.73
	0.3	0.06	0.69	0.005	0.89	0.12	0.94
	3.0	0.09	0.98	0.004	0.94	0.12	0.92
MGM – 1.0	**0.03**	0.10	0.58	0.005	0.99	0.13	0.78
	0.3	0.16	0.77	0.010	0.92	0.24	0.94
	3.0	0.17	0.82	0.011	0.85	0.26	0.95

Appendix 4. Uptake rate constants of MeHg in rapid, gradual and overall uptake by 0.03, 0.15, 0.3 and 3 mg (dw) L^{-1} algal biomasses of cells grown in semi-continuous culture. R^2 in parenthesis.

		Uptake rates (h^{-1})					
MeHg (ng Hg L^{-1}) in Medium	Algal biomass (mg (dw) L^{-1})	Rapid uptake (0-2 h)	(R^2)	Gradual uptake (2-48 h)	(R^2)	Overall uptake (0-48 h)	(R^2)
MGM – 0.5	**0.03**	0.05	0.95	0.001	0.99	0.07	0.83
	0.15	0.06	0.88	0.001	0.99	0.08	0.67
	0.3	0.05	0.94	0.002	0.98	0.10	0.84
	3.0	0.06	0.82	0.001	0.96	0.11	0.60
MGM – 1.0	**0.03**	0.13	0.92	0.003	0.99	0.12	0.76
	0.15	0.10	0.66	0.005	0.96	0.15	0.77
	0.3	0.09	0.72	0.007	0.94	0.17	0.83
	3.0	0.13	0.90	0.008	0.82	0.26	0.93
MGM – 20.0	**0.03**	4.1	0.78	0.07	0.97	3.6	0.64
	0.15	3.0	0.76	0.07	0.97	3.4	0.67
	0.3	2.0	0.91	0.13	0.897	4.0	0.89
	3.0	2.0	0.97	0.12	0.85	5.0	0.92

Appendix 5. Calculated MeHg uptake rate constant (h^{-1}) in 0.03, 0.3 and 3 mg L^{-1} algal biomasses of cells in pseudo steady state conditions over 48 h exposure period. (R^2) in parenthesis.

		Uptake rate (h^{-1}) (R^2)			
		Algal Biomass (mg (dw) L^{-1})			
Medium	MeHg Concentration (ng L^{-1})	0.03	0.15	0.3	3
MGM	0.50	0.07 (0:83)	0.08 (0.883)	0.10 (0.936)	0.11 (0.60)
MGM	1.0	0.12 (0.76)	0.15 (0.77)	0.17 (0.83)	0.26 (0.93)
MGM	20.0	3.6 (0.64)	3.4 (0.67)	4.0 (0.90)	5.0 (0.92)