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INFLUENCE OF UVB RADIATION ON THE CAPACITY OF DISSOLVED ORGANIC CARBON TO PROTECT FRESHWATER PHYTOPLANKTON FROM METAL TOXICITY

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ABSTRACT

Water samples from the Raisin River, Grand River, Lake Simcoe and St. Lawrence River were irradiated in the laboratory for up to 10 d under fluorescent lamps with maximum irradiance in the ultraviolet B waveband (at 310 nm). These waters were chosen to represent a range of dissolved organic carbon (DOC - 30.3, 17.4, 4.4 and 1.9 mg C L^{-1}, respectively). Changes in toxicity of Cu, Zn, Co, Pb, Ni and Cd to *Pseudokirchneriella subcapitata* in response to irradiation depended on sample source and on the metal tested. In the highest-DOC sample (Raisin River), IC_{50} of Cu, Zn, Co and Pb decreased by 30%, 78%, 54% and 56%, respectively, after 10 d UVB, but no change was observed for Ni and Cd. Fewer significant changes in metal toxicity were measured in samples containing lower DOC. DOC concentrations declined only ~ 20% in all samples. DOC fluorescence declined in all samples, but at a faster rate in low-DOC than high-DOC water. Similarly, absorbance per mg C decreased in all samples, and this effect was more pronounced in low-DOC than high-DOC waters.

Newington Bog water (35.5mg C L^{-1}) was irradiated for 20 d under UVB lamps in the laboratory and under natural solar radiation. IC_{50} was significantly reduced with UVB treatment for all four metals tested: Pb - 64%; Cu - 63%; Ni - 35% and Cd - 40%. In the solar treatments, after 20 d exposure IC_{50} also decreased for all four metals, but only the decreases for Pb and Cu were statistically significant.

Solar radiation had a greater negative impact on DOC concentration, DOC fluorescence (DOCFL) and UV absorbance per unit DOC than UVB lamps in equivalent exposure time, and increased toxicity of Pb and Cu more than UVB lamps. Further experiments were performed using a second sample of Raisin River water (20.7 mg C L^{-1}) exposed to 20 d artificial UVA and UVB radiation at or below the maximum irradiance of natural solar radiation. Significant decreases in IC_{50} were observed only for Cu (47%) with UVA and Pb (43%) with UVB. DOC was reduced 20% by both treatments. DOCFL decreased 51.5% in the first 5 d of UVA exposure, an effect that was not observed with the UVB treatment. The UVA treatment decreased UV absorbance more at longer wavelengths and over a broader wavelength band than the UVB treatment.

The varying degrees of change in toxicity of the metals tested in this study indicate that some organic metal-binding ligands are more quickly removed or altered than others when exposed to UV radiation. The DOC remaining after irradiation appears to be qualitatively different from the unirradiated DOC.

At the intensities of solar irradiance associated with clear, sunny conditions, UVA alone or in combination with UVB and/or PAR affects DOC concentration, UV absorbance, and toxicity of Cu and Pb more intensely than UVB alone. The much greater irradiance of UVA (and possibly PAR) makes its contribution to the removal and/or alteration of DOC at least as important as the influence of higher energy UVB. Ozone thinning may not have as much of an impact on DOC concentration and UV absorbance as previously thought,

but UVA and PAR penetrate further into the water column than UVB, and may initiate the process of DOC degradation at greater depths, especially in well-mixed systems. Exposure to UV radiation alters DOC and affects phytoplankton growth in waters where metal concentrations are high.

RESUMÉ

Des échantillons d'eau des rivières Raisin, Grand et St. Laurent, ainsi que du lac Simcoe, ont été irradiés dans le laboratoire sur une période allant jusqu'à 10 jours sous des lampes fluorescentes, possédant une irradiation maximum de longueur d'onde dans l'ultraviolet B (à 310nm). Ces eaux contiennent une gamme de concentration de carbone organique dissous (30.3, 17.4, 4.4 et 1.9 mg C L^{-1}). Les résultats ont démontré que les changements de toxicité à *Pseudokirchneriella subcapitata* du Cu, Zn, Co, Pb, Ni, et du Cd, dû à l'irradiation, dépendaient de la source de l'échantillon et du métal analysé. Les échantillons avec les plus hautes concentrations de COD (rivière Raisin) ont démontré une diminution de la CI_{50} du Cu, Zn, Co et du Pb de 30%, 78%, 54% et de 56% respectivement. Aucun changement n'a été observé pour le Ni et le Cd après 10 jours d'irradiation. Des changements moins significatifs de la toxicité des métaux ont été observés dans les échantillons ayant une faible concentration de COD. Les concentrations de COD ont baissé d'environ 20% pour chaque échantillon. De façon générale, tous les échantillons avaient une fluorecence de COD décroissante. Cependant, les eaux avec une basse concentration de COD ont diminué à un taux plus rapide que les eaux avec une

haute concentration de COD. Pareillement, l'absorbance par mg de C a diminué dans chaque échantillon et cet effet était plus prononcé dans les eaux ayant une basse concentration de COD que celles à haute concentration.

Les eaux de la tourbière Newington (35.5 mg C L^{-1}) ont été irradiées pendant 20 jours sous des lampes UVB dans le laboratoire et par radiation solaire naturelle. La CI_{50} pour les quatres métaux analysés a été significativement réduite avec le traitement par UVB: Pb – 64%; Cu – 63%; Ni – 35% et Cd – 40%. Une baisse de CI_{50} des quatres métaux a été observée après une exposition de 20 jours à la radiation solaire naturelle. Toutefois, seules les diminutions du Pb et du Cu ont été statistiquement significatives.

La radiation solaire a eu un impact plus néfaste que les lampes UVB sur la concentration du COD, la fluorescence du COD (CODFL) et l'absorbance UV par unité de COD, pour des temps d'exposition équivalents. La radiation solaire a aussi causé une augmentation dans la toxicité du Pb et du Cu plus importante que les lampes UVB. Des expériences additionnelles ont été faites, en utilisant un deuxième échantillon d'eau de la rivière Raisin (20.7 mg C L^{-1}). Cet échantillon a été exposé pendant 20 jours à de la radiation UVA et UVB artificielle et à une radiation solaire naturelle. Des baisses significatives de la CI_{50} ont été seulement observées pour le Cu (47%) avec UVA et pour le Pb (43%) avec UVB. La concentration de COD a été réduite de 20% par les deux traitements. CODFL a baissé de 51.5% pendant les premiers 5 jours de l'exposition à radiation UVA. Cet effet n'a pas été observé avec le traitement avec UVB. La baisse d'absorbance UV était plus

importante à grande longueur d'onde et sur une gamme de longueur d'onde plus large pour le traitement UVA que pour le traitement UVB.

Le degré de changement dans la toxicité des métaux analysés indique que certains ligands organiques sont plus rapidement retirés ou modifiés que d'autres lorsqu'ils sont exposés à la radiation UV. Les vestiges du COD après l'irradiation semblent être qualitativement différentes du COD non-irradié.

Aux intensités de la radiation solaire associée à des conditions claires et ensoleillées, l'UVA seule, ou en combinaison avec l'UVB et/ou « PAR », affecte la concentration de COD, l'absorbance UV et la toxicité du Cu et du Pb de façon plus considérable que l'UVB seul. L'irradiation plus importante de l'UVA (et possiblement du PAR) rend sa contribution à l'enlèvement et/ou l'altération du COD au moins aussi important que l'influence de la plus haute énergie de l'UVB. L'amincissement de l'ozone n'a peut être pas un impact aussi important sur la concentration du COD et l'absorbance UV que l'on avait pensé auparavant. L'UVA et le PAR pénétrent plus bas dans la colonne d'eau que l'UVB et peuvent initier le processus de dégradation du COD à de plus grandes profondeurs, surtout dans les systèmes bien mélangés. Une exposition à radiation UV modifie le COD et affecte la croissance du phytoplancton dans les eaux où la concentration de métaux est haute.

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LIST OF ABBREVIATIONS

DGM	dissolved gaseous mercury
DOC	dissolved organic carbon
DOCFL	DOC fluorescence
DOM	dissolved organic material
HMW	high molecular weight
IC_{50}	concentration causing 50% growth inhibition
LMW	low molecular weight
NMR	nuclear magnetic resonance
PAR	photosynthetically active radiation
POC	particulate organic carbon
QSU	quinine sulfate units (units of DOCFL)
RR1	Raisin River sample taken in June, 2001
RR2	Raisin River sample taken in January, 2002
UVA	ultraviolet-A
UVB	ultraviolet-B
WHAM	Windermere Humic Aqueous Model

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION TO THESIS

The research examined dissolved organic carbon (DOC) concentrations, optical properties and metal-binding properties after exposure to UV radiation, and compared observed changes to these properties with changes in metal toxicity to phytoplankton. The literature review provides background information for the research project, including metal toxicity in algae, an overview of DOC and its importance in aquatic ecosystems, changes to DOC caused by UV radiation and other environmental factors, and an assessment of the appropriateness of algal bioassays as a research method.

Unpolluted natural freshwater usually contains only traces of heavy metals such as Cu, Cd, Zn, Pb, and Ni, derived from sources like weathering processes, volcanic activity and erosion of soils and sediments. Natural background concentrations, however, are altered by anthropogenic inputs of metals from activities such as mining, smelting, sewage disposal, burning of fossil fuels, chemical industries and other types of manufacturing activities. Although production of some heavy metals (e.g. Pb) has been curtailed, the use of others (e.g. Ni) are on the increase, and some metals (e.g. Cd) are highly persistent in the environment (Yu, 2001).

Relatively low concentrations of metals cause observable toxic effects in algae. Phytoplankton, as the principle primary producers in aquatic ecosystems, constitute the

base of freshwater aquatic food webs. Algae tend to bioconcentrate heavy metals from the water column, accumulating them in their tissues (Macfie and Welbourn, 2000). Toxicity to phytoplankton therefore poses a double threat to ecosystems: first, by reducing phytoplankton populations that serve as a food source for aquatic species of higher trophic levels, and second, by bioconcentrating metals in the surviving phytoplankton, facilitating their uptake by other species.

Algae can also be viewed as a proxy for the overall health of aquatic systems. If an aquatic environment is polluted with metals to the extent that phytoplankton populations are diminished or phytoplankton species composition is significantly changed, it is likely that the same metals are producing injurious effects in some animals, as well. Although metal toxicity varies from species to species (Borgmann and Ralph, 1983), algae has been shown to be highly sensitive to heavy metals (Lewis, 1995).

Dissolved organic carbon (DOC) in freshwater systems is generally viewed as beneficial to phytoplankton growth, not only because it attenuates harmful UV radiation, but also because it can ameliorate metal toxicity by binding metals. Peatlands (bogs) are a primary source of DOC in fresh waters (Dillon and Molot, 1997 a). The destruction of wetlands, therefore, can be expected to reduce concentrations of DOC, altering the chemistry of downstream waters. To further exacerbate this situation, ultraviolet-B (UVB) radiation is known to photooxidize DOC, reducing its protective capabilities. With the progressive thinning of the ozone layer, UVB radiation is increasing in intensity at the earth's surface.

Global warming reduces the amount of water available to transport DOC into rivers and lakes, and acidification removes DOC (Schindler and Curtis, 1997). These seemingly unrelated, anthropogenically induced environmental problems may jointly pose a threat to primary production in freshwaters.

1.2 LITERATURE REVIEW

1.2.1 Metal Toxicity in Phytoplankton

Yu (2001) defines "heavy" metals as those that are "nutritionally nonessential" and that have a density greater than 5 g/cm^3. The distinction between heavy or toxic metals and nutrient metals is somewhat blurred, however, by the fact that several heavy metals, including Cu, Zn and Co, play a nutritional role at trace concentrations. For the purposes of this paper, the term "heavy metal" will be used to refer to those metals that cause toxicity in phytoplankton at relatively low concentrations, whether or not they may also be nutritive at trace levels.

1.2.2 Importance of Metal Speciation

Two concepts regarding metal toxicity to aquatic organisms stand out as being widely accepted:

1) Metal toxicity in aquatic organisms is strongly correlated with concentrations of free ions of heavy metals, as predicted by the free-ion model of trace metal interactions with aquatic biota (Morel, 1983).

2) In the presence of metal-binding ligands, metal toxicity is ameliorated because free metal ions become bound in inert complexes that are not bioavailable (Parent et al., 1996; Starodub et al., 1987; Sunda and Lewis, 1978).

Some workers have challenged these concepts by showing that metals can be toxic when bound to organic or inorganic ligands (Parent et al., 1996; Tubbing et al., 1994), and that ions other than the free ion can also be toxic to phytoplankton (Parent et al., 1996). Nonetheless, no studies were found to show that free heavy metal ions are not toxic. They are much more heavily implicated in metal toxicity than other chemical species of most heavy metals. A notable exception to this generalization is mercury, which forms highly toxic organocomplexes (Yu, 2001).

1.2.3 Interactions of metals with algal cell surfaces

In order for phytoplankton to be affected by metal toxicity, metal ions must first interact with the cell surface. Ligands on phytoplankton cell surfaces have a strong affinity for metal ions, as they are endowed with a variety of functional groups that have the capacity to bind metals, e.g. carboxylic, amino, thio, hydroxo and hydroxy-carboxylic groups. The affinities of heavy metals for ligands on cell surfaces, however, has been shown by Xue et

al. (1998) to be somewhat reduced in fresh waters due to competition from high concentrations of Mg^{2+} and Ca^{2+} (Xue et al., 1998). Mandal et al. (2000 b) have shown that free Ni^{2+} ion and other labile nickel complexes are significantly increased in the presence of excess Ca^{2+}, Mg^{2+}, Na^{+} or K^{+} because these major cations outcompete Ni^{2+} for binding sites on DOC. The increase in bioavailable nickel was reflected in bioassay toxicity tests with the freshwater green alga *Pseudokirchneriella subcapitata.*

1.2.4 Metal-metal competition

Competition between metals for binding sites with chelators and on algal cell surfaces has frequently been observed, and competition between toxic and nutrient metals is considered by some researchers to be a mechanism of major importance in environmental heavy metal toxicity (Sunda and Huntsman, 1996). Following extensive work with both marine and freshwater phytoplankton, Sunda and co-workers (Sunda and Huntsman, 1996; Sunda and Huntsman, 1998 a; Sunda and Huntsman, 1998 b; Sunda and Lewis, 1978) have determined that heavy metals such as Cu, Cd and Zn interfere with uptake of nutrient metals, most notably Mn^{2+} which is essential for the functioning of photosystem II and detoxification of superoxide radicals. Such interference is caused by competition between heavy metals and Mn^{2+} for binding with Mn transport proteins on the cell surface. In fact, heavy metals like Cu, Cd and Zn have a greater affinity for binding sites on Mn-transport molecules than does Mn^{2+}. In unpolluted marine waters, Mn^{2+} concentrations are sufficiently superior to those of heavy metal ions that Mn^{2+} outcompetes heavy metals in spite of the latter's higher affinity for transport molecules. Although evidence has not yet

been produced for generalization of this situation to a freshwater context, Sunda and co-workers have conducted similar experiments with the coastal diatom *Thalassiosira pseudonana* (Sunda and Huntsman, 1996) and the green alga *Chlamydomonas* (Sunda and Huntsman, 1998 a) with comparable results, and freshwater concentrations of Mn are roughly three orders of magnitude higher than in seawater (calculations from Sunda and Huntsman 1998 a; Sunda and Huntsman 1996; Crump et al., 1999).

Zhang et al. (1997) determined that Ag, Ba, Cd, Cu and Pb bind to the same types of sites on algal surfaces, with conditional affinity constants decreasing in the order: Pb > Cu > Ag > Cd > Ba. Hg binds to a different type of site, and its adsorption to algal surfaces can be enhanced by the presence of Ag^+ (Zhang et al., 1997). In contrast to most studies that relate binding of metals to cell walls with displacement of protons, these authors propose that heavy metals may instead replace Na^+ at low concentrations, or Mg^+ and Na^+ at higher concentrations.

Yu (2001) mentions competition for binding sites on algal cell surfaces between the following ions: Pb^{2+} and Ca^{2+}; Cd^{2+} and Zn^{2+}; Cd^{2+} and Ca^{2+}; Ni^{2+} and Ca^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+} or Zn^{2+}. Bartlett et al. (1974) showed that cadmium seems to inhibit copper toxicity in *P. subcapitata*. Starodub et al. (1987) observed antagonistic interactions between Cu, Zn and Pb, except in the case of Zn and Pb, which behaved additively. According to Xue et al. (1998), metal ions tend to bind to strong binding sites first; once these are saturated,

metal ions will bind to lower affinity sites. As a result, cell surface loading of metals results in lower affinity of metals for the cell surface.

1.2.5 Importance of pH

Metal toxicity is strongly influenced by pH, although there seems to be no consensus around the conditions under which pH increases or reduces metal toxicity to phytoplankton. For example, Campbell and Stokes (1985) divided metals into two groups, according to the tendency for the toxicity of each metal to be directly or inversely related to pH. Their model predicts that 1) the toxicity of "Type I" metals, e.g. Cu, Cd and Zn decreases with decreasing pH, as a result of increased protonation of binding sites on algal surfaces, and 2) "Type II" metals, such as Pb, Al and Hg, show more severe toxicity with increased acidity because concentrations of the most toxic chemical species of these metals are higher at low pH. This model is supported for Type I metals by findings from Peterson et al. (1984) who verified that Cu and Cd were less toxic at low pH and pointed out that, in addition to occupying uptake sites on algal surfaces, H^+ also determines both metal speciation and complexation, metal precipitation and metal-metal interactions. On the other hand, experiments conducted by Starodub et al. (1987) with Cu, Zn and Pb showed that these metals, both alone and in combination, were more toxic at lower pH, i.e. displayed Type II behaviour. Of the three metals studied, only Pb is categorized by Campbell and Stokes as a Type II metal - Cu and Zn are Type I metals. Gensemer (1991) determined that Al^{3+} behaved more like a Type I than a Type II metal, as its toxicity was apparently ameliorated by higher $[H^+]$ in an acidophilic diatom *Asterionella ralfsii* var.

americana. Further, Parent et al. (1996) showed that a drop in pH from 6 to 4 brought about decreased membrane permeability and increased growth inhibition as a result of Al^{3+} toxicity in the green alga *Chlorella pyrenoidosa*.

Starodub et al. (1987) speculated that changes in pH may bring about changes in conformation of metal binding sites on cell surfaces, or alter membrane potentials thereby changing patterns of metal uptake. Macfie et al. (1994) found that higher concentrations of H^+ afforded some protection from metal toxicity, and that this effect was more important than the effect of pH on metal speciation. Gensemer et al. (1993) observed that at very low pH, trace metals can even become limiting.

Transport of metal ions into algal cells has been attributed to passive diffusion down a concentration gradient (Zhang et al., 1997; Xue et al., 1998). Alternatively, heavy metals may enter cells by facilitated diffusion aided by transport proteins whose normal role it is to ensure sufficient intracellular levels of nutrient metals (Sunda and Huntsman, 1996; Sunda and Huntsman, 1998 b). When concentrations of heavy metals are unusually high, as in polluted environments, intracellular systems for regulation of Mn^{2+} increase intracellular concentrations of heavy metal ions when extracellular Mn^{2+} concentrations are low, and reduce intracellular concentrations of Mn^{2+} to damagingly low levels while attempting to diminish levels of heavy metal ions (Sunda and Huntsman, 1996). Similar patterns have been found in Mn- and Zn-uptake systems; Cu inhibited uptake of nutrient concentrations of Zn, reducing growth rate (Sunda and Huntsman, 1998 a). (Zn acts as a

nutrient metal at low concentrations and as a heavy metal at high concentrations.) Similarly, high [Zn^{2+}] inhibits Co uptake in the coccolithophore *Emiliania hyxleyi* (Sunda and Huntsman, 1998 a).

1.2.6 Toxic Modes of Action

Because they are soluble in water, heavy metals are readily absorbed into living tissue where they impair the functioning of biomolecules. In general terms, the toxic mode of action of metals involves interference with the correct functioning of transport proteins, as described above, and enzymes, whose catalytic role in biochemical reactions is made possible by virtue of their conformation. "Cofactors" - generally nutrient metals such as K^{+}, Na^{+}, Mg^{2+}, Mn^{+}, Ca^{2+}, Fe^{2+}, and even Cu^{2+} and Zn^{2+} in very low quantities - become part of enzyme molecules, changing their configuration and enabling them to function. Chemical similarities between metal ions can result in intracellular metal-metal competition, with heavy metals being substituted for nutrient metals as enzyme cofactors causing enzymes to malfunction. Alternately, a metal ion may bind to an enzyme's active site, blocking it and preventing it from performing its biochemical role (Yu, 2001).

1.2.7 Toxic Effects

A range of toxic effects of heavy metals have been observed in algae in response to elevated concentrations of toxic metals. Some examples are listed in Table 1.1.

1.2.8 Detoxification mechanisms

Algae typically produce exudates that act as chelators, reducing metal toxicity (e.g. Starodub et al., 1987). Xue et al. (1998) found, however, that the affinity of copper for the filtered exudates of *Chlamydomonas rheinhardii* is considerably less than its affinity for ligands on cell surfaces. On the other hand, binding capacities of exudates may differ among algal species and for different metals. Murphy et al. (1976), for example, showed that when hydroxamate siderochromes produced by the cyanobacterium *Anabaena flos-aquae* were introduced into the medium of *Scenedesmus basiliensis*, the siderochromes chelated iron to the extent that growth of *Scenedesmus* was suppressed. Growth rates recovered only after the concentration of iron in the medium was increased to overcome the chelating capacity of the *Anabaena* siderochromes. This example illustrates the possibility that different algal species produce exudates that correspond to their specific needs, and may also act as weapons in a sort of chemical warfare that allows one species to dominate a particular environment.

The toxicity of some heavy metals may be reduced in algae due to their strong affinity for binding with ligands on the cell wall. Macfie and Welbourn (2000), for example, compared metal toxicity in both walled and unwalled strains of *Chlamydomonas rheinhardii* with metal binding to cell surfaces and intracellular metal concentrations for Cd, Co, Cu and Ni, to determine whether increased binding to the cell wall could account for increased metal tolerance. Their findings were inconsistent for different metals: while Co and Ni toxicity was reduced by binding to cell walls, Cu and Cd toxicity was not.

In addition to extracellular mechanisms, algae also use intracellular detoxification mechanisms. For example, plants and algae are known to produce "phytochelatins", which are analogous to metallothioneins, the low-molecular-weight, nonenzymatic proteins in animals that bind metals such as Zn and Cd. Phytochelatins are sulfur-rich nonprotein polypeptides that form mercaptide complexes with heavy metals (Yu, 2001). Sunda and Huntsman (1996) demonstrated the existence in *Thalassiosira pseudonana* of an intracellular inducible efflux pump that releases Cd-phytochelatins. The same authors point out that a closely related species of coastal diatom, *Thalassiosira weissflogii*, possesses a similar mechanism. Mehta and Gaur (1999) observed proline accumulation in *Chlorella* exposed to metals, and speculated that it acts as a protective agent against lipid peroxidation by complexing metal ions inside the cell or by reducing or sequestering harmful radicals.

1.3 DISSOLVED ORGANIC CARBON (DOC)

1.3.1 Terminology

Efforts by scientists of different specializations to analyze and describe organic substances in water has led to the invention of a myriad of terms. Examples include "dissolved organic carbon" (DOC), "humic substances", "aquatic humic substances" and others, all of which are intended to refer to "dissolved organic material" (DOM) or some fraction thereof.

The term "dissolved organic material" (DOM) refers to "the largest pool of organic material in the water column" (McKnight and Aiken, 1998). Since DOM is commonly measured as dissolved organic carbon (DOC) (Curtis and Schindler, 1997), the term "DOC" is frequently used instead of DOM. For the purposes of this paper, this convention will be followed.

The word "dissolved" differentiates DOC from other fractions (for example, particulate organic carbon, or POC), and implies that this fraction of organic material will pass through a 0.45μm filter, but the type of filter used is a matter of some contention. In the 1950's, 0.45 μm Millipore membrane (cellulose acetate or nitrate) filters were commonly used. More recently, Whatman 0.45 μm glass fibre filters (GFC and GFF) have become more widely used (by, for example, the Department of Fisheries and Oceans in Experimental Lakes Area research and by the Ministry of Environment at their Dorset facility), but they have nominal pore sizes of 0.8 to 1.2 μm and allow as much as 98% of bacteria to pass through. With advances in filter manufacturing, however, researchers are once again returning to membrane filters such as Poretex or Nucleopore, which employ laser technology to cut more precisely-sized pores (Lean, pers. comm.).

1.3.2 DOC described

The complexity of DOC makes it very difficult to define, however some degree of consistency in functional groups can be observed among samples of DOC from widely varying situations (McKnight and Aiken, 1998). For example, DOC molecules are large

polymers that vary in molecular weight from roughly 1,000 to over 10,000 Daltons (Curtis, 1998). They contain both aliphatic and aromatic carbon structures, to which are attached a variety of functional groups, the most abundant of which are carboxyl and phenolic groups. It is these side-chains that give DOC its characteristic solubility in water, thus setting it apart from organic carbon in soils, which is only sparingly soluble in water.

Precursor materials and the biological pathways involved in degradation (McKnight and Aiken, 1998), as well as the physical conditions under which degradation takes place (Curtis and Schindler, 1997) determine the properties of DOC found in a given place and time. The diversity of these materials, pathways and conditions accounts for the complexity of the mixture of organic molecules that collectively make up bulk DOC.

DOC is produced from both autochthonous and allochthonous sources. Autochthonous DOC is derived from organic material originating inside the water body such as algae, macrophytes and aquatic animal tissues, while allochthonous DOC originates outside the water body, i.e. from terrestrial plant and animal material. The various components of DOC each have their own "life history" which is reflected in their composition and structural characteristics. Terrestrial DOC, for example, is relatively high in aromatic structures, low in nitrogen and optically dense (coloured) (Curtis, 1998; McKnight and Aiken, 1998), whereas autochthonous DOC is richer in nitrogen and more transparent (Curtis, 1998). Bulk DOC in any water body is a mixture of DOC from autochtonous and allochthonous sources from catchment runoff, groundwater and upstream water inflows.

Terrestrial DOC is the most important component in freshwater systems, and is primarily derived from decaying plant material in organic soils. Degradation involves multi-stage processes involving fungi, microorganisms, photochemical reactions and polymerization of small monomers into large polymers. Although many attempts have been made to model these processes, no single model can encompass all of the possible formation pathways (McKnight and Aiken, 1998).

Peatlands are the largest source of terrestrial DOC (Dillon and Molot, 1997 a). Anoxic, acidic, saturated peat soils slow decomposition processes, resulting in the accumulation of organic carbon. As the large biomass of organic carbon gradually decays, high concentrations of DOC are leached to streams.

Molot and Dillon (1997 b) found that stream DOC derived primarily from "recently fixed carbon" in peatlands, rather than from mineral soils. Schindler and Curtis (1997), however, specify that DOC in eastern Ontario streams and groundwater (that has previously passed through wetlands) is likely over 100 years old except when young carbon (less than 40 years old) is flushed from catchments in stormflows.

Attempts to characterize DOC have led to many diverse approaches to its analysis. Curtis (1998) sums these up as follows:

> "The quality of DOM is typically characterized by chemical and related optical properties. For example, DOM is characterized by its affinity to adsorb to synthetic resins, its elemental composition, its isotopic composition and 'age' (^{14}C activity), the density of different functional groups and double bonding, and spectral attenuation of light and subsequent fluorescence. These qualitative properties have been linked to different sources of DOM (allochthonous and autochthonous), and to in situ transformation."

Three ways of categorizing the various components of DOC merit further discussion, as they are often used in studies described in the literature reviewed. One divides DOC into humic substances and other simpler compounds, then separates humic substances into fulvic acids and humic acids (e.g. Mandal et al., 2000 b). Another uses ^{13}C-NMR spectroscopy to determine relative proportions of carbon belonging to specific functional groups (e.g. Clair et al., 1996; Perdue, 1998). A third divides DOC into its coloured and colourless fractions. Each of these is discussed below, in turn.

According to the first method mentioned above, "humic substances" are defined to be organic acids, heterogeneous mixtures of relatively high molecular weight biogenic molecules, characterized by their yellow or brown colour (McKnight and Aiken, 1998). *Aquatic* humic substances are divided into fulvic acids, humic acids and humin, fulvic acids being soluble at any pH, humic acids only at pH > 2 and humin being insoluble at any pH (McKnight and Aiken, 1998). Humic acids and fulvic acids are separated by

acidification, by anion adsorption column or anion and cation exchange resins (Clair et al., 1996).

Evidence of the proportional content of fulvic and humic acids in DOC is inconsistent. According to Thurman (1985), humic substances can make up as much as 90% of DOC in brown-water lakes, and fulvic acids make up 85-90% of dissolved humic substances, with humic acids comprising the remaining 10-15%. Volk et al. (1997) determined that humic substances accounted for 72-78% of the DOC pool in White Clay Creek, Pennsylvania. McKnight and Aiken (1998) report that fulvic acids generally make up roughly 40-60% of DOC in most aquatic systems, and that the non-fulvic-acid portion consists of hydrophilic low molecular weight compounds, synfulvic acid and hydrophobic and hydrophilic organic neutrals. Thurman (1985), lists, in addition to fulvic and humic acids, hydrophilic acid and simple compounds including carbohydrates, carboxylic acids, amino acids and hydrocarbons.

Attempts to describe DOC on the basis of humic acid and fulvic acid content do not provide much information that is useful from a structural standpoint and the methods used to separate these fractions have the undesirable side-effect of modifying them (Clair et al., 1996). Advances in nuclear magnetic resonance spectroscopy have helped to rectify this situation by providing a means of determining relative concentrations of carbon belonging to aliphatic, aromatic, carbohydrate, carboxyl and carbonyl groups. According to Clair et al. (1996), ^{13}C crosspolarization, magic angle spin NMR (^{13}C CP/MAS NMR or ^{13}C NMR

for short) spectroscopy is non-destructive and provides "well resolved, chemically informative, semi quantitative spectra from which the percentage of carbon atoms in various functional groups can be estimated." McKnight and Aiken, (1998) present a comparison of different means of analysis, and note that "although the relative abundance of carbon types may vary, the same bands are present in the ^{13}C-NMR spectra for all samples of humic substances from many environments", providing "a means of discriminating between humic and nonhumic material in aquatic ecosystems and of assessing the origin, fate, and reactivity of aquatic humus."

Using ^{13}C NMR spectroscopy, Clair et al. (1996) analyzed water from four brownwater sites in Nova Scotia, and found that the highest proportion of carbon in DOC was aliphatic (~ 40 - 50%), followed by carbohydrate C (~ 25 - 40%), then carboxylic C (~ 10 - 25%), and lastly, aromatic C (usually < 10%). Seasonal variations were observed, and will be discussed in the section on factors affecting quantity and quality of DOC, below. Similarly, Perdue (1998) used ^{13}C NMR spectroscopy to analyze DOM and DOC in water from the Suwannee River, Georgia, and was able to determine that DOC accounted for approximately 51.6% of DOM, and that aliphatic, aromatic, carboxylic and "excess" C made up ~ 45.6%, ~ 31.0 %, ~ 11.7 % and ~ 11.6 % of DOC, respectively.

Molot and Dillon (1997) examined colour, DOC and mass balances in lakes and streams in central Ontario over 12 years. The chemical composition of the coloured fraction of DOC is unknown. Concentration of coloured DOC is positively correlated with total DOC

concentration and extent of peatlands in the catchment, and it has been shown to be preferentially removed or photobleached (Molot and Dillon, 1997). It is thought to be the coloured portion of DOC that is responsible for its ability to attenuate UV radiation. This is one of the most important aspects of DOC, and will be examined in detail in the next section.

1.3.3 Importance of DOC to aquatic ecosystems

DOC is a critically important part of aquatic chemistry for at least two reasons: it absorbs and attenuates harmful UV radiation, and it complexes heavy metals, making them less bioavailable to aquatic organisms.

Solar radiation in the UVB spectrum (290-320 nm) is detrimental to aquatic organisms, as it causes DNA lesions that can lead to coding errors, ultimately resulting in dysfunction, such as base pair substitutions and frameshifts. DOC absorbs UV radiation in freshwater ecosystems and therefore protects aquatic organisms from the damage UVB can cause (Scully & Lean, 1994). Lean (1998 b) determined that 99% of UVB radiation was absorbed within 0.32 m depth in Ranger Lake, Ontario, which contains about 6 mg DOC/L. In a survey of 11 ponds and lakes, 90% of UVB was found to be attenuated within < 1 to 15 m; this measurement dropped to 12 to 60 cm for humic systems. Crump et al. (1999) investigated UVB penetration in frog ponds near Peterborough containing 7 - 20 mg DOC/L, and found that 99% of UVB radiation was attenuated in the top 10-20 cm. Declining DOC levels cause exponential increases in the ratio of UVB to PAR

penetration, demonstrating that DOC controls depth of UVB penetration (Lean, 1998 b). In fact, Jerome and Bukata (1988) showed that DOC in natural waters attenuates UVB to a greater extent than does the ozone layer, but the extent of UVB protection is likely closely linked to DOC concentration. According to Schindler et al. (1996), "a 10% reduction in DOC in a lake which initially has a concentration of 250 μM will increase the UV exposure of freshwater organisms by more than the projected decreases in stratospheric ozone."

Organic ligands in DOC are widely considered to play an important role in protecting aquatic organisms from metal toxicity by chelating metal ions in inert complexes, (Borgmann and Ralph, 1983; Sunda and Lewis, 1978) thereby attenuating toxicity. The chelating properties of DOC make it a critical factor in the regulation of metal cations, but it does not bind all metals in the same manner and with the same affinity. Baeyens et al. (1998), for example, estimated that in water from the Scheldt estuary (The Netherlands), which contained less than 10 mg DOC/L, 86-99% of Cu, 90-96 % of Zn and 10-35% of Cd was complexed by organic ligands.

According to Leenheer et al. (1998), metal binding with humic substances was shown to depend on pH, ionic strength, concentrations of metal ion and humic ligands, competition between metals, "heterogeneity of metal binding functional group distributions in humic molecules, and size, shape, and phase differences between different humic molecules." Leenheer et al. also determined that Cu^{2+}-binding ligands with log K values near 5 were

more important than other ligands with higher conditional stability constants due to the former's much larger concentrations. They hypothesized that oxygen-containing functional groups, such as carboxyl groups, are likely situated in closer proximity in fulvic acids causing conformational changes to occur upon metal binding and thus involving the fulvic acid molecule as a whole in metal binding, rather than specific functional groups. Powell and Fenton (1996) showed that fulvic acids have lower binding strength for copper than do humic acids; although fulvic acids have more -COOH groups, the few -COOH groups in humic acids are involved in up to 34% of strong metal binding, compared with only 12-23% of -COOH groups in fulvic acids. These findings are supported by Mandal et al. (2000 b) who determined that the greater abundance of negatively charged sites on the larger molecules of humic acids make them stronger polyelectrolytes.

As with algal cell surfaces, competition between metals is an important aspect of metal binding on DOC. Mandal et al. (2000 a and 2000 b) demonstrated that concentrations of nickel in the form of free Ni^{2+} ion and labile nickel complexes increase in the presence of excess Ca^{2+}, Mg^{2+}, Na^{+} and K^{+} due to shielding of negatively charged binding sites on DOC by the major cations. The effect is a net release of labile nickel to the water column. Similarly, Riseng et al. (1991) observed that changes in Al resulted in changes in Fe, Cu, Zn, Co and Mn free ion concentrations in solution, presumably due to differential affinities for chelating agents in the medium.

Chelation can be either beneficial or harmful to phytoplankton, depending on the concentrations of the chelator and of metals in the medium. As one example of the beneficial impact of DOC, Sunda and Lewis (1978) observed that inhibition of cell division in *Monochrysis lutheri* decreased with increased additions of Newport River water containing 22 mg DOC/L. On the other hand, at very high concentrations of chelating substances not only heavy metals but also nutrient trace metals may be complexed and may even become limiting to phytoplankton growth. At low concentrations, chelators release metals that have nutrient qualities for algae (Riseng et al., 1991; Gensemer et al., 1993) but may also release heavy metals into solution.

DOC may influence phytoplankton health in other, less direct ways, for example: by binding inorganics that could otherwise complex nutrients such as PO_4; influencing pH tolerance in algae by changing the lability of trace metals or other nutrients (Gensemer et al., 1993; Parent et al., 1996; Wehr et al., 1998); or by competing with phytoplankton for light (Lean, 1998 b). Parent et al. (1996) found that fulvic acids interact directly with the algal surface, resulting in increases in algal membrane permeability (which counteract decreases in permeability caused by exposure to Al^{3+}) and increased availability of P.

Complex interactions between heavy metals, DOC and nutrient metals may also have an indirect effect on phytoplankton production. For instance, Petersen (1982) showed that *Scenedesmus quadricauda* has greater sensitivity to copper than to zinc, and suggests that

Zn toxicity is a result of displacement of Cu on chelating agents in the medium by Zn, rather than as a result of direct toxic action by Zn.

1.3.4 DOC concentrations in freshwaters

DOC is ubiquitous in freshwaters, but different concentrations can be found in different locations. Relative DOC concentrations determine the vulnerability of aquatic ecosystems to UVB and, possibly, of heavy metals. As a general rule of thumb, DOC levels decline as water progresses from wetlands to streams, rivers and lakes.

The amount of DOC exported from catchments depends largely on the organic content of catchment soils and contact time with water. These factors in turn reflect climatic conditions, surface geology, slope, soil productivity, moisture content and permeability (Curtis, 1998). DOC is highest where wetlands constitute a large proportion of the catchment area (Dillon and Molot, 1997 b) and lowest for locations of high elevation, high latitude or desert (Curtis, 1998).

Values for DOC concentrations in different types of water bodies vary considerably from study to study. Thurman (1985) gives values of 20 to 400 mg DOC /L for bog water, 2 to 25 mg DOC /L for river water and 2 to 30 mg DOC/L for lake water. Curtis (1998), however, gives values of 10 to 50 mg DOC/L for wetlands, which contain the highest levels of DOC (compared with rivers, streams, and lakes), and 0.5 to 3.0 mg DOC/L for alpine lakes, which are at the low end of the range. These values support the findings of

Rasmussen et al. (1989), who showed that the amount of colour in lakes was highest for small, shallow headwater lakes with large, flat catchments and rapid water turnover and lowest for larger deep lakes with small, steep catchments and slow turnover. By way of explanation, these authors note that both the thickness of upper organic soil horizons (which contain the most DOC) and loading of humic matter to lakes are negatively correlated with watershed slope and that the proportion of humic loading is also directly related to the ratio of drainage area to lake area.

1.3.5 Factors affecting the quantity and quality of DOC

Over time, concentrations of DOC can change within a given water body. Long-term changes are generally related to anthropogenic activities, and short-term/seasonal variations occur naturally.

Since wetlands are the principal source of DOC in freshwaters, their destruction reduces DOC concentrations. Wetlands are destroyed both directly by human development projects, and indirectly by increases in temperature due to global warming. As temperatures rise, precipitation decreases, evaporation increases, runoff decreases, water levels decline, wetlands dry up and streamflows diminish (Schindler and Curtis, 1997; Schindler et al., 1997; Clair and Ehrman, 1996). The end result is that less DOC is being produced and transported from wetlands to rivers and lakes.

Clear-cut logging has been shown to temporarily increase DOC concentrations, particularly where the ratio of cut area in the watershed to lake size (surface area or volume) is large, but DOC concentrations decreased significantly with time after the clear-cut event (Carignan et al., 2000).

Acidification of water leads to the destruction of DOC in fresh waters (Schindler and Curtis, 1997). Acid rain is an obvious contributor to this problem, but global warming is also implicated in situations where lake sediments have previously sequestered high concentrations of sulfur from activities such as smelting. As water levels decline, littoral sediments become exposed and reduced S is oxidized. Subsequent precipitation can then mobilize the oxidized S into the water body, where it forms sulfuric acid that destroys DOC (Yan et al., 1996). DOC removal may be caused by the direct effects of acidification and/or the indirect effects of increased concentrations of Al^{3+} which saturates DOC causing flocculation (Yan et al., 1996; Lean, 1998 a).

Within a water body, DOC quantity and quality are modified by in situ processes. Autochthonous production and evapoconcentration of DOC influence the overall balance of bulk DOC in the positive direction. On the other hand, dilution by precipitation, flocculation/ sedimentation and mineralization to CO_2 by microbial activity or photolysis decrease DOC concentrations. All of these processes become more important with increased water residence time (Curtis, 1998), but on the whole, losses are greater than gains.

It is well known that UVB radiation brings about photolytic reactions in DOC, eventually causing photobleaching (e.g. Lean, 1998 b; Sunda and Huntsman, 1998 b). Photolytic reactions between UVB radiation and DOC result in the mineralization of carbon and its release to the atmosphere in the form of CO_2 and CO (Dillon and Molot, 1997 b; Lean, 1998 a). Exposure to sunlight breaks large, refractory DOC molecules down into more labile, light molecular weight (LMW) compounds, the major ones being formaldehyde, acetone, glyoxal, methylglyoxal, glyoxylate and pyruvate (Lean, 1998 b; Molot and Dillon, 1997). Mineralization can then happen either as a direct result of photooxidation, or indirectly as DOC is photooxidized into LMW organic compounds that are subsequently degraded biotically by bacteria (Molot and Dillon, 1997).

Terrestrial DOC, which is more highly coloured than autochthonous DOC, has a shorter residence time in lakes than does bulk DOC (Curtis and Schindler, 1997). Schindler et al. (1996) attribute the loss of colour to increased photobleaching and photodegradation of DOC as water residence time in a lake increases. Once coloured DOC is lost, the remaining DOC has lower photoreactivity. As more and more of the photoreactive portion of DOC is removed, water becomes progressively clearer and the remaining DOC becomes less and less able to attenuate UV radiation. The end result is deepening thermoclines, euphotic zones and zones of critical UV exposure for aquatic organisms (Curtis and Schindler, 1997). This phenomenon has particularly serious repercussions for clear lakes that have low concentrations of DOC and long water retention times (Curtis

and Schindler, 1997; Lindell, 1996). When DOC drops below 2 mg DOC/L, the depth of UVB penetration rapidly increases (Lean, 1998 b).

Evidence for removal of DOC by photooxidation can be found in the seasonal variations in chemical structures and coloured fractions. Sensitivity of DOC to sunlight in a given lake is least at the end of summer and greatest during winter and spring, especially in humic lakes (Lindell, 1996). Lean (1998 b) used DOC fluorescence (DOCFL) as an indicator of photoreactivity and UV absorbance, and observed that the UVB-absorbing fraction of DOC is highest in May, but much lower in June through August, in some cases increasing again in September with inputs of fresh DOC (presumably due to increased precipitation). When exposed to direct sunlight, up to 30% of DOCFL may be lost in a day, whereas DOC measurements do not decrease to the same extent, probably indicating reduced importance in the proportion of UV-absorbing DOC and corresponding increased importance in non-absorbing DOC.

Clair and Sayer (1997) analyzed water from four brownwater sites in Nova Scotia, examining seasonal trends in proportions of "chromophoric" carbon structures. Chromophores are structures that include conjugated double bonds, such as aromatic groups (Lean, 1998 b) and carboxylic functional groups (Thurman, 1985). Clair and Sayer showed an increase in the relative proportions of these groups from June to November. At first glance this data would seem to contradict most observations on the subject, given associations of colour loss and reduced UVB attenuation over the summer season. It is

very likely, however, that Maritime watersheds would receive increased precipitation towards the end of summer and into the fall, i.e. well before the November samples were taken, and that fresh DOC would have been transported into the sampling locations.

Photooxidation of DOC does not present problems when associated with natural seasonal changes in hydrologic and climatic factors. Global warming and ozone depletion, on the other hand, have more serious implications for the long term, especially when combined with other anthropogenically induced environmental changes such as acid rain and destruction of wetlands, as described above. In whole-lake experiments conducted over a 20-year period, Schindler et al. (1997) found that a temperature increase of 1.6°C resulted in longer water residence times and a corresponding 15 to 25% loss (or "retention") of DOC concentration. Although the extent to which this temperature rise can be attributed to global warming is debatable, this example shows the potential for significant losses of DOC if temperatures continue to rise.

In acidified lakes, DOC retention was even more elevated (Schindler et al, 1997). In fact, Schindler et al. (1996) speculate that some changes in aquatic ecosystems observed after acidification may in fact be more appropriately attributed to the increased impact of UV radiation.

Although only 17% of photooxidation of DOC to inorganic carbon can be attributed to degradation by UVB (Lindell, 1996), studies of UVB interactions with DOC are

increasingly warranted as it is this spectrum that will increase in intensity as a result of stratospheric ozone depletion.

1.3.6 Implications for phytoplankton

Based on the above discussion, it is clear that the beneficial properties of DOC are rather short-lived in terms of protection from UVB and possibly from heavy metals, as well.

There are a few examples in the literature of the links between UV radiation and the metal binding properties of UVB, for instance, Sunda and Lewis (1978) exposed Newport River water to 4 h of intense UV radiation, which removed >99% of the DOC and brought about a 2-3 order of magnitude decrease in copper complexation, and Sunda and Huntsman (1998 b) reported that euphotic zone concentrations of Cu^{2+} in the central Sargasso Sea (off of Florida) are 20-40 times greater than at depth due to photodegradation of chelating agents.

Only one study was found that links UVB radiation, DOC and metal toxicity in algae. Rahayu (2000) demonstrates that after 10 days UVB irradiation of Raisin River water (initially containing 24.4 mg DOC/L), DOC is reduced by roughly 20%, whereas absorbance at 265 nm drops by about 52%. These findings are in line with other studies cited earlier which show that UVB absorbance and colour are lost at a greater rate than the overall concentration of DOC. Rahayu then used the same water in algal bioassays and found that the concentration of metal to bring about 50% growth inhibition (IC_{50}) of

Pseudokirchneriella subcapitata dropped from ~ 90 μg Cu^{2+}/L to ~ 3 μg Cu^{2+}/L as UVB irradiation time increased from 0 to 10 days. She observed the same trend, although a less dramatic decline in IC_{50}, with Ni^{2+}. In other words, with increased exposure to UVB, DOC concentrations drop slightly, while copper and nickel toxicity increase significantly, and absorbance at 265 nm drops dramatically. Based on these results, when DOC is exposed to UVB radiation, it seems to lose its metal-binding capacity before its carbon structure is photodegraded.

On the other hand, some workers have found that phosphorus, trace metals and other inorganic nutrients that are complexed by fresh DOC are released when it is photodegraded by UVB, to the benefit of primary producers (West et al., 1999; Vinebrooke and Leavitt, 1998; Molot and Dillon, 1997). West et al. (1999) found that growth rates in *Pseudokirchneriella subcapitata* were stimulated by low exposure to UVB in lake water samples containing both high and low concentrations of DOC, and points out that DOC has been associated with both negative and positive effects on phytoplankton but on the whole, high concentrations of DOC were found to have a net protective effect. These findings are also confirmed by Rahayu (2000), who found that *P. subcapitata* introduced to Raisin River that had been previously irradiated for five and ten days grew 37% and 36% more, respectively, than in controls with non-irradiated water.

The process of photodegradation not only bleaches DOC and releases metal ions into the water column, but photochemical reactions also lead to production of harmful byproducts,

such as hydrogen peroxide, carbon monoxide and free radicals (Vinebrooke and Leavitt, 1998; Lean, 1998 a; West et al., 1999). Kaczmarska et al. (2000) determined that clear water phytoplankton are subjected to far less chemical stress than brown water phytoplankton, due in large part to high production levels of H_2O_2 in brown waters. H_2O_2 has a relatively long half-life and may be distributed deeper in the water column by vertical mixing.

It is clear from the above discussion that the impact of UVB on DOC may have both detrimental and beneficial implications for phytoplankton. In addition to its direct injurious effects on phytoplankton, UVB radiation also reacts with DOC to change water chemistry, reducing beneficial qualities and increasing bioavailability of metals and other toxic materials. At the same time, it releases nutrients into the water column. However, the scarcity of studies conducted to date on the interactions of UVB, DOC and heavy metal toxicity in algae indicates that more research is required.

1.4 ALGAL BIOASSAYS

This study employed a series of bioassays with the green alga *Pseudokirchneriella subcapitata* (formerly known as Selenastrum capricornutum) as the test organism to determine the influence of UV irradiation on the capacity of dissolved organic carbon to protect algae from metal toxicity.

Algal bioassays are useful tools for evaluating metal toxicity in phytoplankton, provided that appropriate precautions are taken to avoid factors than might otherwise skew results. Environment Canada (1992) employs algal bioassays using *P. subcapitata* as a test organism, and lists the following advantages of this method: increased efficiency compared with algal bottle tests; small sample volume; smaller space requirements; reduced contamination from reuse of glassware; greater ease of preparation, and by extension, larger numbers of tests and replicates are possible in a given period of time.

On the other hand, the possibility of transport of volatile substances between microplate wells, reduced toxicity of test substances due to filtering, variability in concentrations of dissolved organic material and nutrients in test waters, pH shifts, adsorption of contaminants to microplate surfaces, contamination by micro-organisms and difficulties in measuring results may lead to unreliable results. It is possible, however, to control test conditions to minimize or eliminate most of the above concerns (Environment Canada, 1992).

Algae are generally easy to culture under laboratory conditions, and are susceptible to metal toxicity at low concentrations of metals (Guy and Kean, 1980). For example, in experiments with the green alga *Chlamydomonas reinhardtii*, Macfie et al. (1994) calculated an EC_{50} for algal growth of 0.4 μM or 25.4 μg/L for copper, 0.1 μM or 11.2 μg/L for cadmium and 2.1 μM or 123.3 μg/L for nickel. Starodub et al. (1987) ranked Cu, Zn and Pb according to their toxicity to photosynthetic activity in *Scenedesmus*

quadricauda, in the following order: Cu (EC_{50} = 100 µg/L) > Zn (EC_{50} = 250 µg/L) > Pb (EC_{50} = 2700 µg/L), in the absence of a complexing agent. To their further credit, "Because phytoplankton have short life cycles, they respond quickly to environmental change and their diversity and density can indicate the quality of their habitat." (Lewis, 1995).

Pseudokirchneriella subcapitata is a unicellular, non-flagellated, crescent-shaped, freshwater green alga (Chlorophyceae), that can be found throughout North America. According to Environment Canada (1992), *P. subcapitata* is moderately sensitive to toxic substances, and Lewis (1995) points out that algae are highly sensitive to metals and cationic surfactants. *P. subcapitata* grows rapidly, is easy to culture in the laboratory, and is relatively easy to count because it does not tend to clump (Environment Canada, 1992). Guy and Kean (1980) determined that it does not have a perceptible influence on speciation of metals (i.e. by means of excretion of chelating exudates or other chemical change agents).

1.5 RATIONALE, HYPOTHESES AND OBJECTIVES

There is evidence in the literature reviewed that at the intersection of three anthropogenically derived environmental problems - thinning of the ozone layer, reduced DOC concentrations and heavy metal pollution of aquatic environments - lies the potential for significant reduction of primary productivity in freshwater ecosystems. Both UVB

radiation and heavy metals are injurious to phytoplankton, on which depends the healthy functioning of aquatic ecosystems. DOC clearly plays an important role in regulating metal speciation, and also in attenuating UVB radiation, and is itself regulated by UVB.

Little is known regarding the precise nature of the mechanisms linking together UVB, DOC and metal toxicity in phytoplankton. The study by Rahayu (2000) on this subject, although provocative, is based on a single experiment, and no other papers were found that specifically examined the relationships between these physical, chemical and biological factors.

If DOC is removed by UV radiation, and/or if its metal binding capabilities are reduced, then metals will be released from DOC with irradiation, making them more readily available for uptake by aquatic biota. The principal hypothesis of this study is that with increasing exposure of natural freshwater to UV radiation, toxicity of metals to phytoplankton increases. DOC concentration, DOC fluorescence and UV absorbance are easily measurable parameters that allow characterization of DOC without risk of altering its composition or structure. A further objective of this study was to determine whether the degree of change in these parameters could predict any observed changes in metal toxicity after a given period of UV exposure.

The results of this study are relevant to decision-making regarding the generation and disposal of metal-containing waste products. They also contribute to the growing body of

knowledge surrounding protection of wetlands and reduction of ozone-depleting emissions and greenhouse gases.

Table 1.1 Toxic effects of heavy metals on algae

Toxic effect	Reference
Negative feedback Mn^{2+} regulation mechanisms can exacerbate intracellular Mn deficiency brought on by inhibition of Mn uptake by heavy metal ions.	Sunda and Huntsman (1998 a)
Cu, Cr, Ni and Zn cause oxidative stress in algae (*Chlorella vulgaris*), which in turn brings about rupture of cell membranes and loss of K^+ due to lipid peroxidation.	Mehta and Gaur (1999)
$CuSO_4$ added to cultures of *Monochrysis lutheri* reduced cell division rates and increased cell volume.	Sunda and Lewis (1978)
Copper toxicity in *Pseudokirchneriella subcapitata* causes extended lag growth phase.	Bartlett et al. (1974)
Treatment of algal cultures with mixtures of heavy metals causes reduction in cellular concentrations of Mg^{2+}, Ca^{2+} and K^+.	Zhang et al. (1997)
Inorganic Al decreases cell membrane permeability; once inside the cell, Al interrupts metabolism of phosphorus.	Parent et al. (1996)
Inhibition of cell division by Al in two acido-tolerant green algae, possibly due to initial death of algal cells and/or time required for surviving cells to adapt to higher Al concentrations.	Claesson and Tornqvist (1988)

2.0 INCREASED METAL BIOAVAILABILITY FOLLOWING ALTERATION OF FRESHWATER DISSOLVED ORGANIC CARBON BY ULTRAVIOLET-B RADIATION EXPOSURE

2.1 INTRODUCTION

DOC is a critically important part of freshwater chemistry for two reasons: a) it absorbs and attenuates harmful UVB radiation, thereby controlling depth of UVB penetration (Lean, 1998 b), and b) it complexes heavy metals, and transports them into lakes and rivers from the headwaters at concentrations in excess of that predicted by solubility; and 3) it makes heavy metals less bioavailable to phytoplankton (e.g. Starodub et al., 1987; Sunda & Lewis, 1978).

Dissolved organic carbon (DOC) is a complex mixture of large organic polymers ranging in molecular weight from roughly 1,000 to over 10,000 Daltons (Curtis, 1998). It is operationally defined as the fraction of organic matter that passes through a 0.45 um filter. DOC contains both aliphatic and aromatic carbon structures to which are attached a variety of functional groups, including carboxyl and phenolic groups, which are capable of binding metals. Allochthonous DOC gives a yellow-brown colour to natural fresh water.

Both UVB radiation and heavy metals are injurious to phytoplankton, on which depends the healthy functioning of aquatic ecosystems. Metal toxicity in aquatic organisms is

thought to be strongly correlated with concentrations of free ions of heavy metals, as predicted by the Free Ion Activity Model (FIAM) of trace metal interactions with aquatic biota (Morel, 1983). Toxicity to phytoplankton poses a double threat to ecosystems: first, by reducing phytoplankton populations that serve as a food source for aquatic species of higher trophic levels, and second, by bioconcentrating metals in the surviving phytoplankton, facilitating their uptake by other species.

DOC does not bind all metals in the same manner and with the same affinity. For example, the stability constants (log k values) for complexes of Cu, Ni, Co, Zn, Pb and Cd with citrate, a weak organic ligand, are 7.2, 6.7, 6.3, 6.1, 5.4 and 5.0, respectively (Morel & Hering, 1993). Since DOC is also a weak organic ligand, the stability constants for metal-DOC complexes can be expected to differ among various metals.

The amount of DOC exported from catchments depends largely on the organic content of catchment soils and contact time with water. These factors in turn reflect climatic conditions, surface geology, soil productivity, moisture content and permeability (Curtis, 1998), slope and drainage basin area to lake area ratio (Rasmussen et al., 1989). DOC is highest where wetlands constitute a large proportion of the catchment area (Dillon and Molot, 1997a) and lowest for locations of high elevation, high latitude or desert (Curtis, 1998).

Peat bogs (wetlands) are a primary source of DOC in fresh waters (e.g. Dillon and Molot, 1997a). Their destruction by a variety of anthropogenic activities reduces DOC concentrations, altering the chemistry of downstream waters. The end result is that less DOC is being produced and transported from wetlands to rivers and lakes (Schindler and Curtis, 1997; Schindler et al., 1997; Clair and Ehrman, 1996) where it can serve as a sunscreen (Schindler et al., 1997, Yan et al., 1998).

DOC absorbs radiation primarily in the UV range and is photooxidized by UVB radiation. With the progressive thinning of the ozone layer, UVB radiation is increasing in intensity at the earth's surface. Evidence for removal of DOC by photooxidation can be found in seasonal variations in chemical structures and coloured fractions (Clair et al., 1996). Photooxidation of DOC does not present problems when associated with natural seasonal changes in hydrologic and climatic factors. Global warming and ozone depletion, on the other hand, have more serious implications for the long term.

Knowledge of the ways in which DOC is changed with increased UVB exposure is required to assess the potential consequences of greater UVB to primary production in freshwaters. The above factors indicate that the beneficial metal binding properties of DOC may be rather short-lived. This study examines the potential for UVB radiation to reduce the capacity of DOC to protect phytoplankton from heavy metal toxicity. The primary hypothesis to be tested is that with UVB exposure, metal toxicity to phytoplankton increases, and it is expected that this relationship will be more evident for

metals with higher stability constants for binding with weak organic ligands (e.g. Cu, Ni), and less so for those metals that are known not to bind strongly to organic material (e.g. Cd). Further, increases in metal toxicity are expected to be greater in waters containing "fresh" DOC from headwaters than in waters that have been exposed to solar radiation for long periods of time.

2.2 MATERIALS AND METHODS

2.2.1 Water samples

In order to contrast freshwaters containing different DOC concentrations, samples were taken from two brownwater sites and two clearwater sites. The brown water samples were taken at the headwaters of the Raisin River at Monkland, Ontario (45°12' lat.; 75°52' long.) and the headwaters of the Grand River at Riverview, Ontario (44°06' lat.; 80°22' long.). Clearwater samples were taken from Lake Simcoe at Oro Station, Ontario (44°27' lat.; 79°31' long.) and the St. Lawrence River at Morrisburg, Ontario (44°54' lat.; 75°11' long.). The Raisin River flows into the St. Lawrence River 20 km east of Cornwall, Ontario. Wetlands are a common feature in its headwater regions and it flows to the St. Lawrence through regions that are primarily agricultural. The Grand River has its headwaters in the peat bogs west of Dundalk, Ontario and flows southward into Lake Erie through primarily agricultural land interspersed with many small towns and cities that are home to mixed industrial activity. The St. Lawrence River drains the Great Lakes, which have residence times ranging from 191 yr for Lake Superior to 2.6 yr for Lake Erie. Most

of the water flowing down the St. Lawrence originates in Lake Ontario, which has a renewal time of about six yr (US EPA and Government of Canada, 1995). Lake Simcoe is a large, deep lake in southern Ontario roughly 100 km north of Toronto with surface area of 725 km^2 and mean residence time of 7.23 yr (Scott et al., 2001). Thirty-five rivers flow into Lake Simcoe, draining a watershed of 2,848 km^2, which includes the cities of Barrie and Orillia, a number of smaller towns, as well as agricultural lands and an estimated 12,000 cottages (Scott et al., 2001; Lake Simcoe Region Conservation Authority, 2002).

Samples were filtered through 0.45 μm Gelman mixed cellulose ester filters and stored at 4°C prior to use, then irradiated under UVB fluorescent lamps in 2.2 L Teflon bottles (11.5 cm diameter) in the laboratory for five and ten days. UVB irradiance was measured using an OL754 spectroradiometer with dual monochromator (Optronics Ltd.), OL IS470-W submersible sphere, OL 730-7Q-WP waterproof fibre optic probe and operated with OptoLab 754 software. Spectral irradiance is graphed in Figure 2.1. At the outside surface of the bottle (in air) the UVB dose (integrated over 280-320 nm) was 3.90 W/m^2. The dose inside the bottle was measured by cutting a hole in the side of one of the bottles and sealing the probe's sensor inside the bottle with parafilm, then filling the bottle with deionized water. The UVB dose at the inside surface of the bottle opposite the lamps was 1.38 W/m^2. UV absorbance of the deionized water was assumed to be insignificant. Since the integrated UVB irradiance in full sun ranges from approximately 0.80 to over 2.5 W/m^2, the UVB irradiance inside the bottles was approximately half that of maximum summer solar radiation at the earth's surface, although UVB lamps produced some

irradiance at shorter wavelengths than the shortest wavelengths in solar radiation at the earth's surface. Darker, higher-DOC waters attenuated most of the irradiation within the diameter of the bottle, especially for the shortest wavelengths.

After irradiation, bottles were stored in the dark at 4°C. Non-irradiated bottles were kept in the dark at 4°C and used for controls. Subsequent experiments with Grand River water have shown that DOC concentration, DOCFL and UV absorbance in dark controls kept at the temperature of the irradiated bottles vary only slightly from refrigerated samples after 30 d (Winch, unpublished data).

2.2.2 Algal bioassays

Algal bioassays were used to examine changes in metal toxicity in water from all four sources following UVB irradiation. The bioassays were performed in accordance with the Environment Canada (1992) protocol for algal bioassays using the green alga *Pseudokirchneriella subcapitata* (commonly known as *Selenastrum capricornutum*). An axenic culture was obtained from the University of Toronto Culture Collection and cultured in 250 mL Erlenmeyer flasks at 24 ± 2°C using AAP medium (Environment Canada, 1992), renewed weekly under sterile conditions.

EDTA was not used in the tests, which were conducted in Nalge Nunc International 96-well, flat-bottomed polystyrene microplates and incubated for 72 hr in a Converon growth chamber at 24°C under cool white fluorescent lights at 100 mmol m^{-2} s^{-1}.

For each of the twelve water samples (i.e. subsamples of each of the four samples, irradiated 0 d, 5 d and 10 d), separate bioassays were conducted using sample water spiked with Cu^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, Co^{2+} and Pb^{2+}. These metals were chosen to represent a range of stability constants for complexation with weak organic ligands. In each case, a working solution was initially made by diluting 1000 mg/L metal nitrate solutions (purchased from Fisher Scientific) in sample water to obtain the highest metal concentration to be used in the bioassay (maximum concentrations are listed in Table 2.1). Nitrates were used rather than sulfates because nitrate is a relatively weak complexing agent for metals. Concentrations of nitrate added were negligible compared with the concentration of nitrate in the nutrient spike prepared in accordance with the Environment Canada protocol. The highest concentration of metal nitrate used (2000 µg/L Co) would provide 950 µg/L of N, a relatively small amount when compared with the 15.94 mg/L $NaNO_3$ (2630 µg/L N) from the nutrient spike (see Table 2.2). The nutrient spike ensures that nitrates and other nutrients are present in sufficient concentrations in the test medium so that they are not limiting to growth, therefore small additions of N from metal nitrates or pH adjustment would not change the test outcome.

Working solutions were prepared three days prior to the initiation of the bioassays. Each working solution was brought to the same pH as the non-spiked test water using small amounts of 0.1 M NaOH. This adjustment was necessary to compensate for acidification following addition of the reference metal solutions. Working solutions were shaken vigorously and allowed to equilibrate at 4°C until the test date.

To prepare the microplates, each working solution was diluted in 10% increments (to 90%, 80%, 70%, ..., 10%) with more sample water and added to the microplate, as shown in Figure 2.2. The D row in each microplate was used for negative controls, i.e. sample water only with no added metals. Each test well received 200 µL of test water, an additional 10 µL of algal suspension (approximately 2,200 *P. subcapitata* cells suspended in 10 µL of 15 mg/L $NaHCO_3$, in order to produce a final concentration of *P. subcapitata* cells was approximately 10,000 cells/mL), and 10 µL of nutrient spike prepared in accordance with the Environment Canada protocol (1992). The final concentrations of nutrients for each test well are listed in Table 2.2.

pH was spot-checked in controls of test plates before and at the end of the test period. pH rose from approximately 8 to 8.5 over the course of the 72 h incubation, except in St. Lawrence River samples, which dropped from an initial pH of 8.8 to approximately 8.2. Quality control plates were prepared using $Cu(NO_3)_2$ (maximum concentration of 100 µg/L), distilled water and nutrient spike to ensure that the algae were healthy. The last column in each microplate was a blank for the spectrofluorometer that included the test water and nutrient spike but no algae or metals.

After incubation, growth inhibition was determined by reading chlorophyll-a fluorescence with a Molecular Devices Corporation SPECTRAmax GEMINI XS dual-scanning microplate spectrofluorometer, using an excitation wavelength of 460 nm and an emission

wavelength of 680 nm. Overlap with DOC fluorescence is unlikely as the excitation wavelength for DOC fluorescence is 365 nm (Scully et al., 1996).

Calibration curves were established for chlorophyll-a fluorescence in each non-irradiated water sample by diluting a suspension of *P. subcapitata* in sample water and scanning it in the microplate spectrofluorometer. Prior to dilution, the algal suspension was counted manually six times using a hemacytometer to establish the cell concentration. The suspension was then diluted in series (dilution factor = 50%) with more sample water. Each test well received 200 µL of test water, 10 µL of buffer (15 mg/L $NaHCO_3$) and 10 µL of nutrient spike (as described above for the bioassay preparations), in order to ensure that the final suspensions in the microplate wells would contain the same components as a bioassay microplate. The microplate was scanned immediately in the microplate spectrofluorometer. Calibration curves were linear ($r^2 > 0.99$) for all samples.

In order to verify that chlorophyll-a fluorescence provided accurate measures of cell concentration, after 72 h incubation, algae cells in some microplate wells were counted manually six times using a hemacytometer, and these cell counts were plotted against the corresponding fluorescence measurements. Figure 2.3 shows the calibration curves from the dilution series, and data from manual cell counts following bioassays in one brownwater (Raisin River) and one clearwater (St. Lawrence River) sample. Regression analysis of the manual counting data produced similar curves to those obtained from the dilution series, but the variability was greater in the manual data set (Raisin River

$r^2 = 0.82$; St. Lawrence River $r^2 = 0.95$). Given the greater precision of the data generated by chlorophyll-a fluorescence measurements than by manual counting, fluorescence data have been used for calculation of IC_{50} values and estimates of cell concentrations in this study.

Percent growth inhibition, as determined from chlorophyll-a fluorescence values, was plotted against log10 concentration for each metal. The linear section of each curve around IC_{50} was isolated and analyzed by replicated (linear) regression. A minimum of three concentrations (five replicates each) was used in all regressions. IC_{50} was calculated from the resulting regression equation and 95% confidence limits were determined by inverse prediction. IC_{50} values are considered significantly different where 95% confidence intervals do not overlap (i.e. $\alpha = 0.05$).

2.2.3 DOC concentration

DOC content was determined using an IO Analytical 1010 total organic carbon analyzer (persulfate oxidation method), to examine the change in DOC concentration with UVB treatment.

2.2.4 DOC fluorescence

Aromatic groups tend to absorb UV radiation more strongly than aliphatic groups. Since fluorescence requires that radiation be first absorbed, DOC fluorescence should be a better measure of the aromatic proportion of DOC than DOC concentration (Lean, 1998 b).

Aromatic groups are often substituted with oxygen-containing functional groups such as carboxyl and hydroxyl groups that also are important sites for metal binding. Changes in DOCFL after irradiation were monitored in order to determine whether DOCFL is a better predictor of metal binding capacity than DOC concentration. DOC fluorescence of each sample was read using a Turner Designs Model 10 fluorometer, fitted with excitation filters #7-60 and 5G (excitation wavelength 365 nm), emission filter # 47-B and reference filter 2A (emission wavelength 437 nm) (Scully et al., 1996). Quinine sulfate standards were used and all measurements were converted into quinine sulfate units (qsu), where 1 qsu = 1 μg/L quinine sulfate in 0.1 M H_2SO_4.

2.2.5 UV Absorbance

Absorbance was measured on all subsamples for wavelengths 200 to 800 nm using a Varian Cary 100 Bio UV-visible spectrophotometer and quartz cuvettes (pathlength = 1 cm).

2.2.6 Speciation analysis

Background concentrations of anions and cations were measured by ion chromatography for each non-irradiated water sample. For each sample, the maximum concentration of each tested metal spiked into bioassays was added to the measured background concentration to give total metal (e.g. total Cu).

In order to predict concentrations of each metal remaining in solution after binding to DOC, total metal and all background concentrations of other cations and anions were used as input to the Windermere Humic Aqueous Model (WHAM, Version 1.0) software. WHAM is a chemical equilibrium model combined with a simple inorganic speciation code for aqueous solutions, and is designed for use where chemical speciation is dominated by organic matter. Specifically, WHAM employs Humic Ion-Binding Model V as a model of ion-binding by humic substances. This model is based on stability constants gathered from the literature to predict metal binding to DOC.

Total metal concentrations at pH 7.25, ionic strength 0.005 M at 25°C were used as input to WHAM in order to estimate the concentration each metal remaining in solution after binding to DOC. WHAM output (% of each total measured anion and cation remaining in solution), was then multiplied by the total initial concentration of each ion to give concentrations of metals in solution.

In order to predict inorganic speciation of metals not bound to DOC, the WHAM predictions for each metal in each test water were used as input data for MINEQL Version 3.01a. MINEQL is a software package that predicts inorganic speciation, drawing on a database of over 2000 stability constants and taking into account conditions of pH, temperature and ionic strength.

2.3 RESULTS

2.3.1 Algal bioassays

Appendix 1 gives IC_{50} values and their 95% confidence limits for all six metals in all water samples after 0 d, 5 d and 10 d irradiation. Figure 2.4 present trends in IC_{50} for all six metals in all four water samples. IC_{50} values for Cu, Zn and Pb in Raisin River water decreased significantly (95% confidence limits do not overlap) after 10 d UVB exposure by factors ranging from 30% to 78% lower than initial values. IC_{50} for Co decreased by 54%, although the change was not statistically significant. No significant change in IC_{50} was observed for Ni or Cd in Raisin River water for the same exposure period. In the Grand River samples, IC_{50} decreased with increasing UVB exposure for Cu (25%), Ni (35%) and Zn (37%), although the decrease was only statistically significant for Cu, and IC_{50} for Cu decreased after 5 d then increased slightly after 10 d exposure time. IC_{50}'s decreased for four of the six metals in Lake Simcoe water (Cu, Ni, Pb, Co), but the changes were only statistically significant for Pb (76% decrease) after 10 d UVB. In St. Lawrence River water, significant decreases in IC_{50} were observed after 10 d irradiation for Cu (76% decrease) and Ni (56% decrease), but for all of the other four metals, IC_{50} increased significantly after 10 d UVB exposure.

2.3.2 DOC concentration

In the Raisin River samples, DOC concentrations dropped from an initial concentration of 30.3 mg/L to 26.9 mg/L after 5 d irradiation, and 24.2 after 10 d. Grand River DOC

concentration decreased from 17.4 mg/L to 15.4 mg/L after 5 d UVB exposure and 14.5 mg/L after 10 d exposure. Non-irradiated Lake Simcoe water had an initial DOC concentration of 4.4 mg/L, decreasing to 4.0 mg/L after 5 d and 3.4 mg/L after 10 d irradiation. St. Lawrence River water initially contained 1.9 mg/L DOC, decreasing to 1.4 mg/L and 1.6 mg/L after 5 d and 10 d UVB exposure, respectively. All measurements were subject to machine error of ± 0.2 mg/L. The apparent slight increase in DOC in St. Lawrence River subsamples between 5 d and 10 d UVB exposure can, therefore, be attributed to machine error.

Conversion of DOC to $\log_{10}$DOC allows the calculation of a first-order rate constant for DOC loss. Figure 2.5 shows $\log_{10}$DOC concentrations for subsamples from all four locations. No significant difference was found between slopes of $\log_{10}$DOC vs. exposure time ($p = 0.96$), and $r^2 > 0.95$ for three of the four regression lines shown in Figure 2.5. Sample size used in this calculation is small, however (df = 3), so power to detect small differences in slope is low. On the other hand, $\alpha = 0.05$, and $p >> \alpha$, therefore the slopes can be considered equal. R^2 for the St. Lawrence regression was 0.34 due to very low concentrations of DOC in this sample. Dropping the St. Lawrence regression lowers p to 0.38, but does not change the outcome.

The mean slope of the remaining three regressions is -0.0097. The rate constant for DOC

loss in these three samples was computed as:

$$k = 2.303\ (-0.0097) = -0.02234\ d^{-1}$$

$$d[DOC]/dt = -0.02234[DOC]\ (mg\ C\ L^{-1}\ d^{-1})$$

DOC was removed at a rate of ~2% /d. Turnover time, therefore, was calculated as $1/k \approx$ 50 d. This rate constant is applicable to samples initially containing > 4 mg C L^{-1}, and holds true for at least 10 d exposure to UVB radiation at the levels used in this study.

2.3.3 DOC fluorescence

Figure 2.6 shows changes in $\log_{10}$DOCFL with UVB irradiation. Measurements of DOCFL for Raisin River water decreased from an initial value of 152 qsu to 147 qsu after 5 d irradiation and 125 qsu after 10 d irradiation. Similarly, Grand River DOCFL fell from 147 qsu to 125 qsu after 5 d and 119 qsu after 10 d UVB irradiation. DOCFL in both Lake Simcoe and St. Lawrence River water measured 4 qsu at the outset, decreasing to 2 qsu and then to 0 qsu after 5 and 10 d irradiation in the St. Lawrence subsamples; Lake Simcoe subsamples measured 4 qsu after 5 d irradiation and 2 qsu after 10 d irradiation.

Differences between the slopes of $\log_{10}$DOCFL vs. exposure time were statistically significant ($p = 0.04$) when compared across all four samples. When the clearwater samples were eliminated, however, p increased to 0.94, therefore a rate constant was

calculated from the mean slope of the darkwater samples (-0.0089).

$$k = 2.303(-0.0089) = -0.02050\ d^{-1}$$

$$d[DOCFL]/dt = -0.02050[DOC]\ (mg\ C\ L^{-1}\ d^{-1})$$

In these samples, DOCFL removal took place at a rate of ~ 2% /d, and would be completely removed in 50 d. Based on the data from this study, the above rate constant can be applied to removal of DOCFL from water containing $17 < DOC < 31$ mg C L^{-1} and holds true for at least 10 d exposure to UVB radiation at the levels used in this study.

2.3.4 UV absorbance

In all four samples, UV absorbance decreased with increasing UVB exposure. Slopes of ln abs vs. wavelength (see Figure 2.7) after 10 d UVB exposure for Raisin River water decrease from an initial -0.0170 to -0.0153, and for Grand River water, from -0.0175 to -0.0159. Slopes of clearwater ln abs vs. wavelength plots are steeper and increase with exposure to UVB (-0.0229 to -0.0273 for Lake Simcoe; -0.0199 to -0.0232 for St. Lawrence River).

Figure 2.8 is a plot of ln(abs/DOC), which allows a comparison of the quality of DOC remaining after irradiation, with respect to its ability to absorb in the UV range per unit DOC (Morris et al., 1995). Raisin River and Grand River water absorbance per mg C was higher than abs/mg C in St. Lawrence or Lake Simcoe water. Surprisingly, St. Lawrence

water had higher abs/mg C than Lake Simcoe water, although the bulk DOC was lower in the St. Lawrence sample. In all samples, absorbance/DOC decreased with irradiation, but this effect was more pronounced in the low-DOC samples than in the dark waters.

2.3.5 Metal speciation

For all four water samples, MINEQL predicted that Zn, Cd, Co and Pb in solution (i.e. not bound to organic matter) would be present in solution as free metal ion (Zn^{2+}, Cd^{2+}, Co^{2+} and Pb^{2+}). Both Ni and Cu, however, form hydroxide complexes within the range of conditions of pH, temperature, concentration and ionic strength of the bioassays (see Figure 2.9). The presence of these aqueous complexes would reduce the concentrations of Cu^{2+} and Ni^{2+} around the pH of the bioassays, thereby reducing the lability of the free metal ions.

2.4 DISCUSSION

2.4.1 DOC characterization - concentration, fluorescence and absorbance

It was expected that DOC concentrations would decrease with UVB irradiation, that metal toxicity would be greatest in low-DOC waters, and toxicity would increase following exposure to UVB, particularly for high-DOC waters. Changes in optical properties of DOC were monitored by measuring DOCFL and examining UV absorbance spectra to determine whether the aromatic proportion of DOC remaining after UVB irradiation would change.

The Raisin River and Grand River (brownwater) samples were initially found to be much richer in DOC than the Lake Simcoe and St. Lawrence River (clearwater) samples, likely due to the fact that the clear water has been exposed to the sun's radiation for a much longer period than the headwater samples. DOC concentrations decreased by approximately 20% with irradiation in all samples. This is not surprising, given that DOC is known to be photodegraded with exposure to UV radiation (Molot & Dillon, 1997).

DOCFL decreased roughly the same amount (20%) in the brownwater samples as the decrease in DOC concentration. In the clearwater samples, DOCFL decreased much faster than DOC concentration. In clear water, the aromatic fraction of DOC decreases in proportion more quickly than in dark waters. This is confirmed by the absorbance data that shows absorbance per unit DOC to decrease more quickly in St. Lawrence River and Lake Simcoe water than in Raisin River or Grand River water. This effect is probably due to self-shading in the darker waters, and the lower concentrations of DOC and consequently greater penetration of UVB into clear waters.

2.4.2 Metal toxicity

Changes in metal toxicity could not be correlated with changes in DOC concentration, DOCFL or absorbance for any one metal across all four water samples. Twelve of the 24 series of tests conducted in this study (each series including tests on water from a given source irradiated 0, 5 and 10 d) produced evidence of increased metal toxicity with 10 d exposure to UVB. However, trends in IC_{50} were not consistent across all six metals tested,

or across all four water samples. The effect of UVB on metal toxicity, therefore, may depend not only on the concentration of DOC present, but also on the characteristics of DOC in a given water sample, and on the metal being tested.

For example, metal toxicity is likely influenced by inorganic speciation. The Raisin River results indicate that toxicity increased (i.e., IC_{50} decreased) with exposure to UVB, except in bioassays spiked with Ni and Cd. Cd is known to be weakly bound to organic ligands (Morel and Hering, 1993). It is not surprising that Cd showed no significant increase in toxicity after UVB irradiation, since very little of the Cd would be bound to DOC in non-irradiated water, so the amount released with exposure would be relatively small. Nickel, on the other hand, binds strongly to organic ligands. If metal binding capacity of DOC is reduced with UVB irradiation, it would follow that a relatively large increase in concentration of labile Ni would be expected after UVB irradiation. No such trend was observed in this study. Figure 2.9, however, shows that Ni^{2+} is replaced by $Ni(OH)_2$ at pH > 8. Bioassays for Raisin River, Grand River and Lake Simcoe were initiated at ~ pH 8, and pH in the test wells increased to 8.3-8.8 over the course of the 72 h incubation as a result of algal respiration of CO_2. The saturation index of $Ni(OH)_2$ at pH > 8 indicates that some Ni was likely being removed by precipitation rather than remaining in solution as Ni^{2+} or another labile species. Cu forms aqueous hydroxide complexes around the pH of the bioassays. These complexes may be bioavailable, but if not, they would reduce copper toxicity to algae. Both Ni^{2+} and Cu^{2+} may also be removed from solution by sorption to colloidal iron hydroxides.

Equilibration time for Ni is also important. According to Xue et al. (2001), Ni speciation may never reach equilibrium in natural waters due to slow ligand-exchange kinetics. If so, the bioassays would have been conducted before the Ni^{2+} reached equilibrium with the DOC in the subsamples, and the fact that there were relatively fewer binding sites in the UVB exposed water may have been obscured by the process of Ni^{2+} binding to the existing sites as these sites became increasingly saturated. More research would be required to determine whether IC_{50} values for Ni and Cd would decrease significantly with longer UVB exposure.

Rahayu (2000) carried out experiments with Cu and Ni in Raisin River water (initially containing 24.4 mg DOC/L), and found that after 10 d UVB irradiation, DOC was reduced by roughly 20%. IC_{50} for growth inhibition of *P. subcapitata* dropped from 90 μg Cu^{2+}/L to 3 μg Cu^{2+}/L over a period of 10 d UVB irradiation and a similar but less dramatic drop in IC_{50} was observed with Ni^{2+}. The difference between these findings and the observations of this study may be due to the fact that in the study by Rahayu, much smaller volumes of water were irradiated in quartz tubes (2.5 cm in diameter) where they would have received a much higher effective intensity of radiation throughout. In this study, 2.2 L Teflon bottles were used (11.5 cm in diameter), and the dark-colored water would have attenuated most of the UVB radiation within a few cm (self-shading). Crump et al. (1999) measured the depth of 99% UVB attenuation to be 12 cm in pond water containing 19.7 mg/L DOC. It is likely that in the Raisin River water used in this study, which initially contained 30.3 mg/L DOC, 99% of UVB radiation would be attenuated within the

diameter of the bottle. The water would only receive something close to the full intensity of irradiation as it circulated closest to the radiation source. This irradiation method more closely approximates natural circumstances, where UVB is attenuated within the top few cm of high DOC water. The intensity of solar radiation, however, does not deteriorate as rapidly with distance from the source as does the intensity of radiation from lamps in the laboratory. In a natural setting, UVB radiation likely penetrates further into the water column than can be simulated with lamps.

IC_{50} for all metals was much lower in clearwater samples than in brown water. Since the clearwater samples used in this study would have previously undergone extensive exposure to sunlight (mean residence times of 6 yr and 7.2 yr in Lake Ontario and Lake Simcoe, respectivley), the lack of colour and lower ability to mitigate metal toxicity likely reflects the extent to which photodegradation of DOC has progressed.

2.5 CONCLUSIONS

This study shows that as UVB exposure time increases from 0 d to 10 d, DOC concentrations in natural freshwaters decline, and toxicity of some metals to *P. subcapitata* increases. The time intervals used in this study are very short and the intensity of UVB radiation low compared with the exposure of DOC in natural water to sunlight as it moves from peat bogs through streams, rivers and lakes. Given differences observed between IC_{50} values for relatively fresh, high-DOC water from the headwaters of the

Raisin and Grand rivers and much "older" water from Lake Simcoe and the St. Lawrence River, the effects observed in this study are probably many times greater in the natural environment.

Bioassays using other types of freshwater algae and irradiated water are beyond the scope of this study, but numerous published studies show that the growth of many common freshwater phytoplankton is significantly reduced by metal toxicity (e.g. Macfie and Welbourn, 2000; Claesson and Tornqvist, 1988; Petersen, 1982). Destruction of freshwater DOC by UVB irradiation, therefore, may influence primary productivity and species composition. These findings have important implications for metal loading criteria.

It is not possible to determine from this study whether toxicity varies more dramatically with changes in DOC quality than with decreases in DOC concentration, although the results provide an intriguing basis for additional research.

Some of the factors contributing noise to the relationship between algal growth inhibition and DOC quality and quantity include behaviour of individual metals (e.g. formation of hydroxides by Cu and Ni around pH 8) and the source-specific characteristics of DOC in each water sample. Longer irradiation times are necessary to determine more definitively the nature of the relationship between toxicity of different metals, DOC concentration and quality, and UVB irradiation.

Table 2.1 Maximum concentrations of metals used to spike algal bioassays with Raisin River, Grand River, St. Lawrence River and Lake Simcoe water exposed to 0 d, 5 d and 10 d UVB radiation.

Sample	Metals					
	[Cu]	[Ni]	[Zn]	[Cd]	[Co]	[Pb]
Raisin River - 0 d	200 µg/L (3.1 µM/L)	800 µg/L (13.6 µM/L)	200 µg/L (3.1 µM/L)	200 µg/L (1.8 µM/L)	2000 µg/L (33.9 µM/L)	1000 µg/L (4.8 µM/L)
Raisin River - 5 d	200 µg/L (3.1 µM/L)	800 µg/L (13.6 µM/L)	200 µg/L (3.1 µM/L)	200 µg/L (1.8 µM/L)	2000 µg/L (33.9 µM/L)	1000 µg/L (4.8 µM/L)
Raisin River - 10 d	200 µg/L (3.1 µM/L)	800 µg/L (13.6 µM/L)	100 µg/L (1.5 µM/L)	100 µg/L (0.9 µM/L)	500 µg/L (8.5 µM/L)	500 µg/L (2.4 µM/L)
Grand River - 0 d	100 µg/L (1.55 µM/L)	800 µg/L (13.6 µM/L)	100 µg/L (1.5 µM/L)	100 µg/L (0.9 µM/L)	500 µg/L (8.5 µM/L)	500 µg/L (2.4 µM/L)
Grand River - 5 d	100 µg/L (1.55 µM/L)	800 µg/L (13.6 µM/L)	100 µg/L (1.5 µM/L)	100 µg/L (0.9 µM/L)	500 µg/L (8.5 µM/L)	500 µg/L (2.4 µM/L)
Grand River - 10 d	100 µg/L (1.55 µM/L)	800 µg/L (13.6 µM/L)	100 µg/L (1.5 µM/L)	100 µg/L (0.9 µM/L)	500 µg/L (8.5 µM/L)	500 µg/L (2.4 µM/L)
St. Lawrence River - 0 d	50 µg/L (0.79 µM/L)	200 µg/L (3.4 µM/L)	50 µg/L (0.76 µM/L)	50 µg/L (0.44 µM/L)	200 µg/L (3.4 µM/L)	200 µg/L (0.96 µM/L)

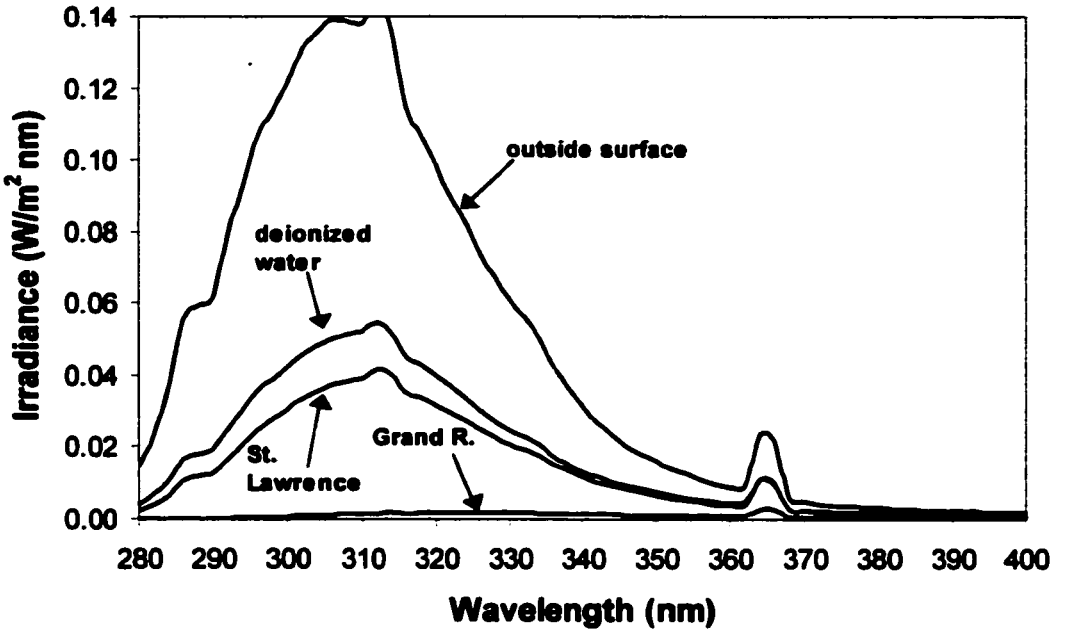
St. Lawrence River - 5 d	50 µg/L (0.79 µM/L)	200 µg/L (3.4 µM/L)	50 µg/L (0.76 µM/L)	50 µg/L (0.44 µM/L)	200 µg/L (3.4 µM/L)	200 µg/L (0.96 µM/L)
St. Lawrence River - 10 d	50 µg/L (0.79 µM/L)	200 µg/L (3.4 µM/L)	50 µg/L (0.76 µM/L)	50 µg/L (0.44 µM/L)	200 µg/L (3.4 µM/L)	200 µg/L (0.96 µM/L)
Lake Simcoe - 0 d	50 µg/L (0.79 µM/L)	200 µg/L (3.4 µM/L)	50 µg/L (0.76 µM/L)	100 µg/L (0.89 µM/L)	200 µg/L (3.4 µM/L)	200 µg/L (0.96 µM/L)
Lake Simcoe - 5 d	50 µg/L (0.79 µM/L)	200 µg/L (3.4 µM/L)	50 µg/L (0.76 µM/L)	100 µg/L (0.89 µM/L)	200 µg/L (3.4 µM/L)	200 µg/L (0.96 µM/L)
Lake Simcoe - 10 d	50 µg/L (0.79 µM/L)	200 µg/L (3.4 µM/L)	50 µg/L (0.76 µM/L)	50 µg/L (0.44 µM/L)	100 µg/L (1.7 µM/L)	100 µg/L (0.48 µM/L)

Table 2.2 Final concentration of nutrients in the test medium used in algal bioassays.

(Adapted from Environment Canada, 1992)

Macronutrient	Concentration (µg/L)	Micronutrient	Concentration (µg/L)
N	2630	B	20.27
Mg	1650	Mn	72.11
Ca	750	Zn	0.98
S	1200	Co	0.22
P	120	Cu	0.003
K	293	Mo	1.8
Na	6880	Fe	20.7
C	1340		

Figure 2.1 Spectral irradiance of UVB lamps.


0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
Irradiance (W/m² nm)
outside surface
deionized water
St. Lawrence
Grand R.
280 290 300 310 320 330 340 350 360 370 380 390 400
Wavelength (nm)

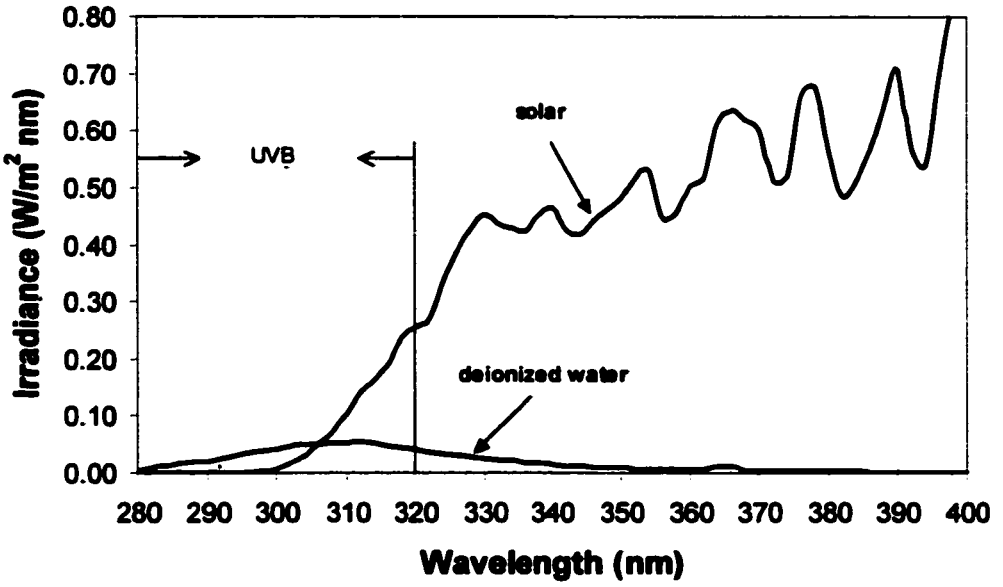
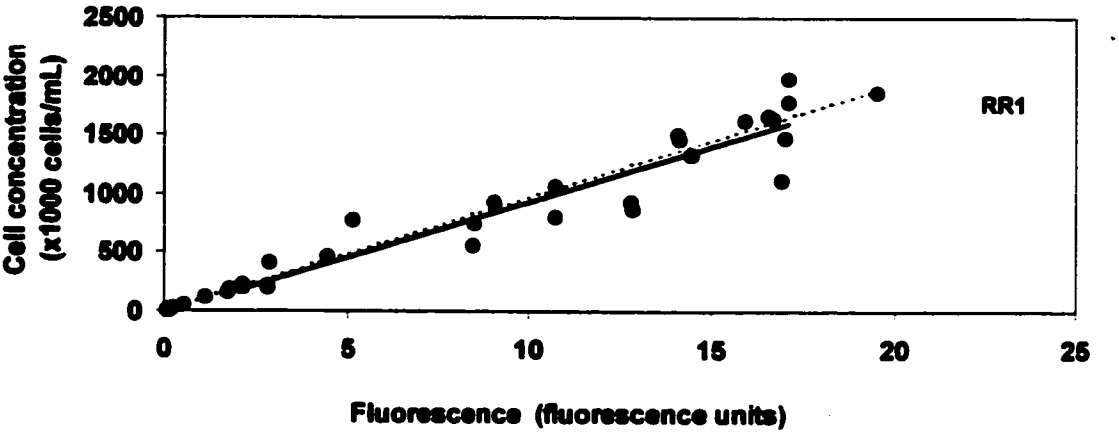

0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Irradiance (W/m² nm)
UVB
solar
deionized water
280 290 300 310 320 330 340 350 360 370 380 390 400
Wavelength (nm)

Figure 2.2 Template for bioassay microplates ("100%" = maximum concentration; C = controls).

	100%	90%	80%	70%	60%	50%	40%	30%	20%	10%	
	100%	90%	80%	70%	60%	50%	40%	30%	20%	10%	
	C	C	C	C	C	C	C	C	C	C	
	100%	90%	80%	70%	60%	50%	40%	30%	20%	10%	
	100%	90%	80%	70%	60%	50%	40%	30%	20%	10%	
	100%	90%	80%	70%	60%	50%	40%	30%	20%	10%	

Figure 2.3 Comparison of cell count estimates from chlorophyll-a fluorescence of a diluted algal suspension (black circles and broken line;) and manual cell counts by hemacytometer from microplate wells at the end of algal bioassays (grey circles and solid line) in Raisin River (RR1) water (manual $r^2 = 0.820$; fluorescence $r^2 = 0.999$) and St. Lawrence River (SLR) water (manual $r^2 = 0.949$; fluorescence $r^2 = 0.999$).


Cell concentration (x1000 cells/mL)
2500
2000
1500
1000
500
0
0
5
10
15
20
25
Fluorescence (fluorescence units)
RR1

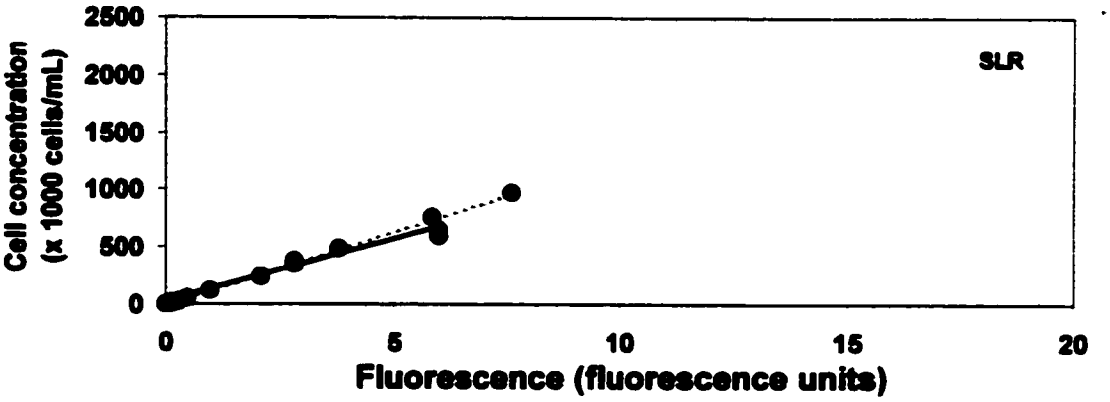
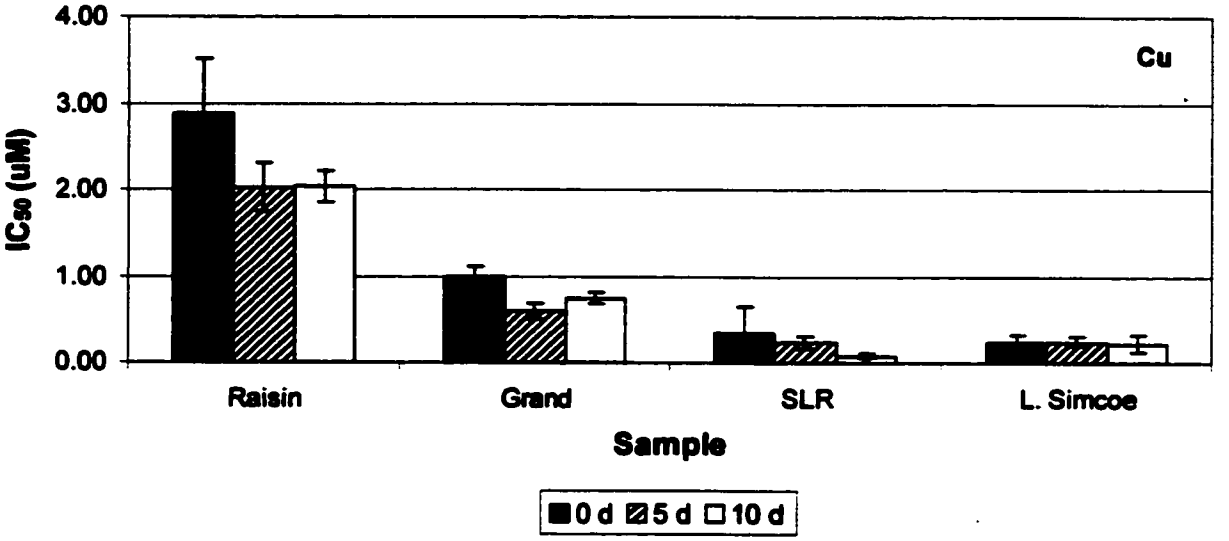
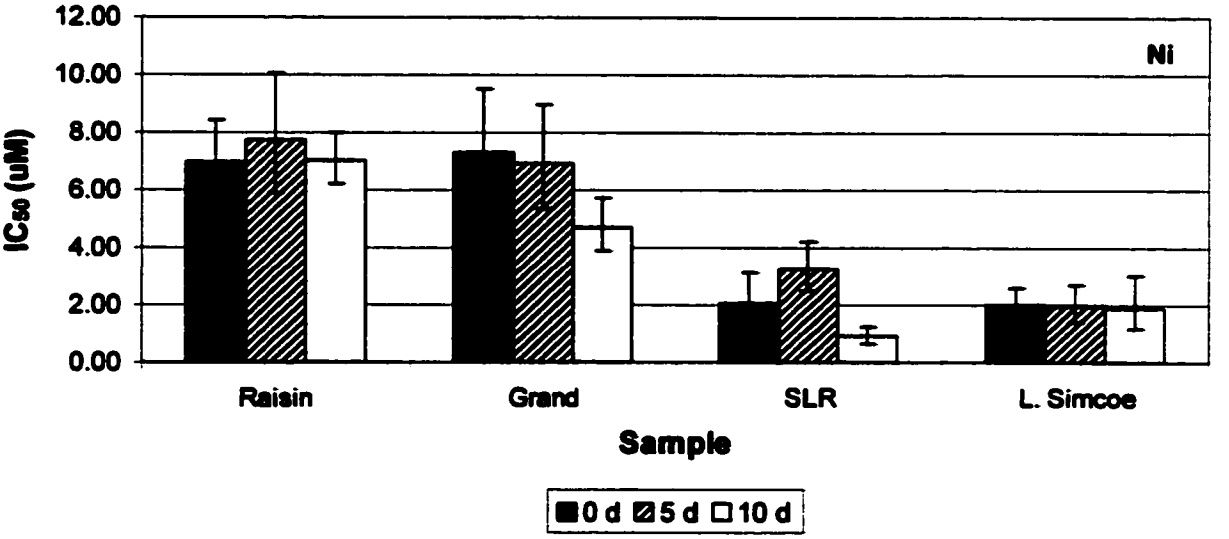
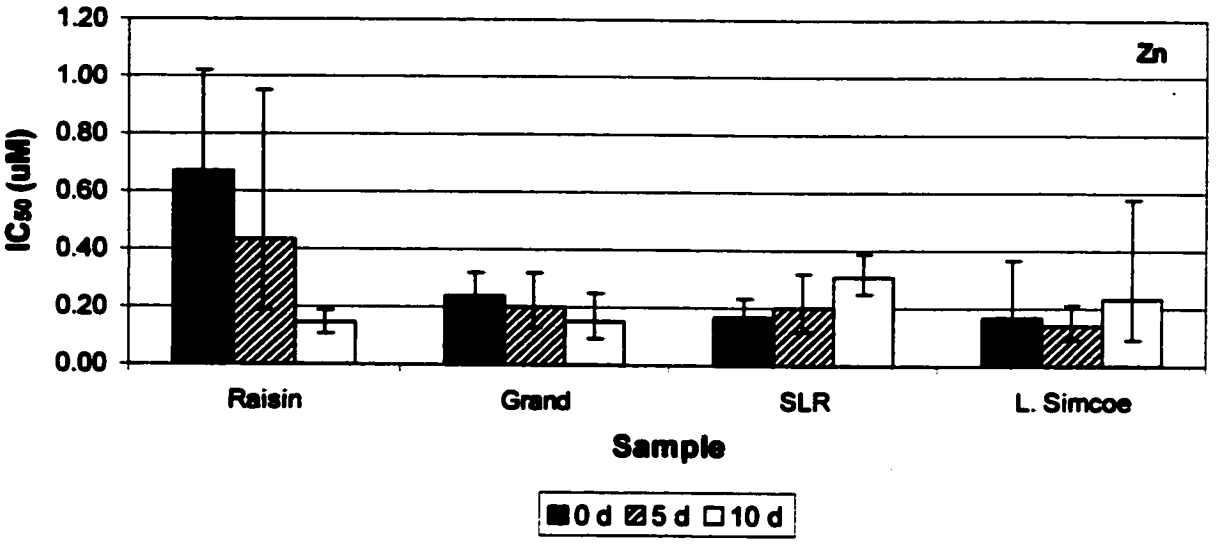
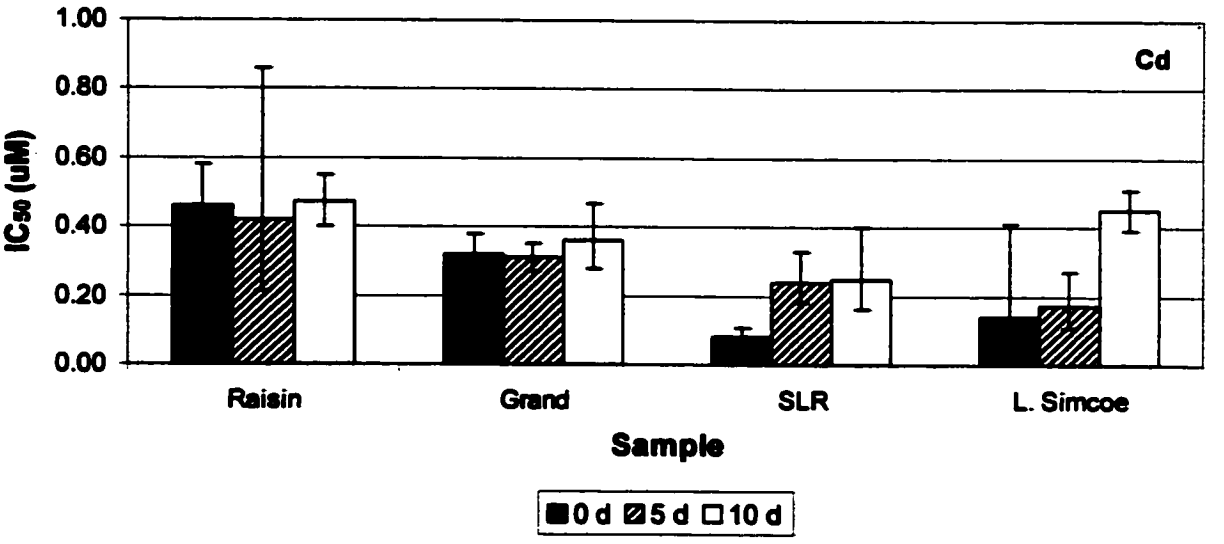

Cell concentration (x 1000 cells/mL)
2500
2000
1500
1000
500
0
0
5
10
15
20
Fluorescence (fluorescence units)
SLR

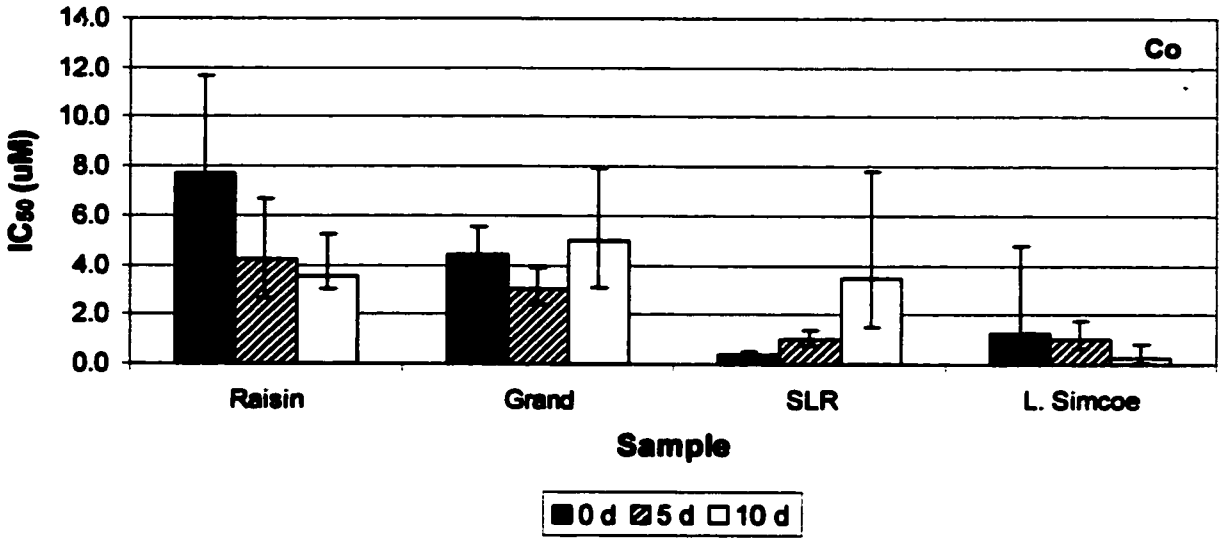
Figure 2.4 IC_{50} (μM) and 95% confidence limits for Cu, Ni, Zn, Cd, Co and Pb in Raisin River (RR1), Grand River (GR), Lake Simcoe (SIM) and St. Lawrence River (SLR) water after 0, 5 and 10 d exposure to UVB irradiation.


Cu
4.00
3.00
2.00
1.00
0.00
IC50 (uM)
Raisin
Grand
SLR
L. Simcoe
Sample
0 d
5 d
10 d


Ni
12.00
10.00
8.00
6.00
4.00
2.00
0.00
IC50 (uM)
Raisin
Grand
SLR
L. Simcoe
Sample
0 d
5 d
10 d


1.20
1.00
0.80
0.60
0.40
0.20
0.00
IC50 (uM)
Zn
Raisin
Grand
SLR
L. Simcoe
Sample
0 d 5 d 10 d


1.00
0.80
0.60
0.40
0.20
0.00
IC50 (uM)
Cd
Raisin
Grand
SLR
L. Simcoe
Sample
0 d 5 d 10 d


Co
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
IC50 (uM)
Raisin
Grand
SLR
L. Simcoe
Sample
0 d 5 d 10 d

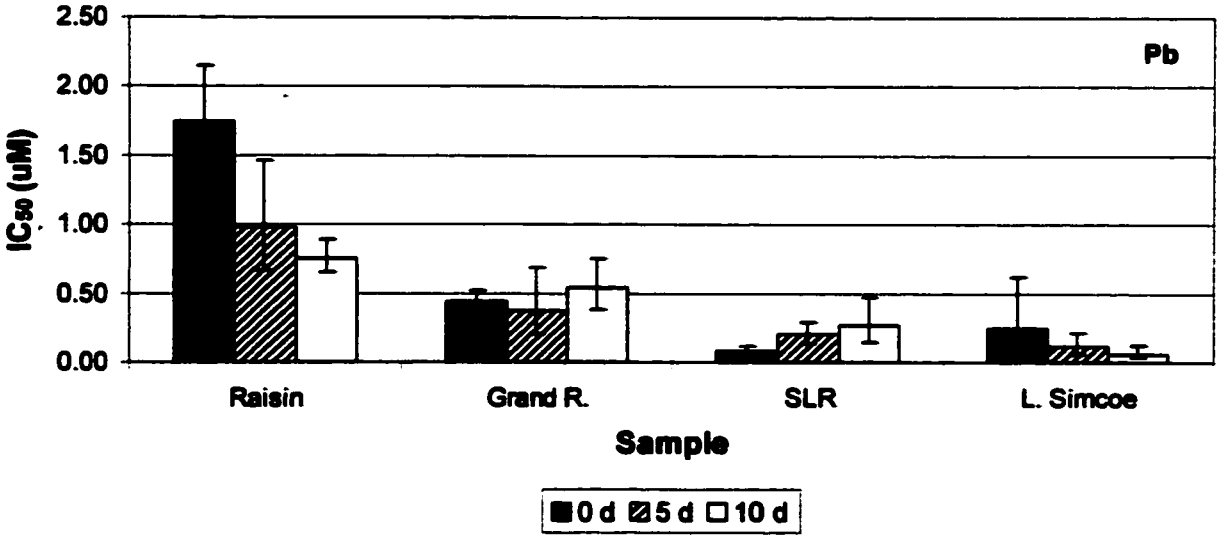

Pb
2.50
2.00
1.50
1.00
0.50
0.00
IC50 (uM)
Raisin
Grand R.
SLR
L. Simcoe
Sample
0 d 5 d 10 d

Figure 2.5 Log_{10}DOC (mg C L^{-1}) in Raisin River (RR1; diamonds), Grand River (squares), Lake Simcoe (triangles) and St. Lawrence River (circles) water after 0 d, 5 d and 10 d UVB irradiation.

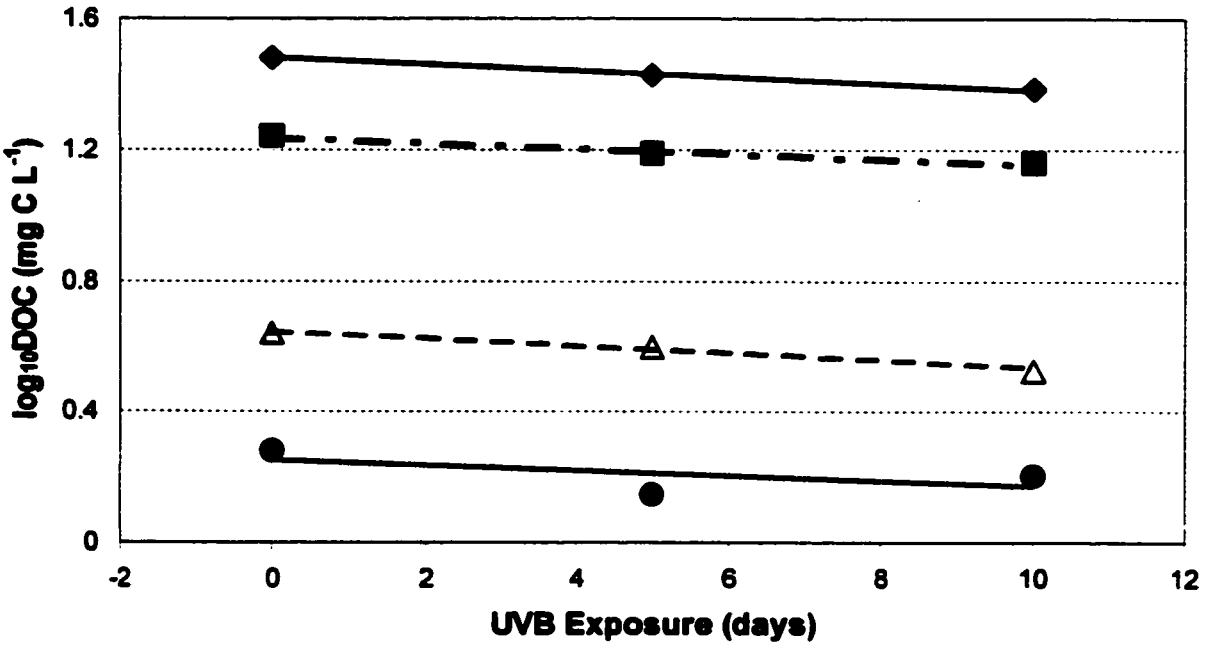

1.6
1.2
0.8
0.4
0
log10DOC (mg C L-1)
-2
0
2
4
6
8
10
12
UVB Exposure (days)

Figure 2.6 Log_{10}DOCFL (DOC fluorescence - qsu; excitation λ = 365 nm, emission λ = 437 nm) in Raisin River (diamonds), Grand River (squares), Lake Simcoe (triangles) and St. Lawrence River (circles) water after 0 d, 5 d and 10 d UVB irradiation.

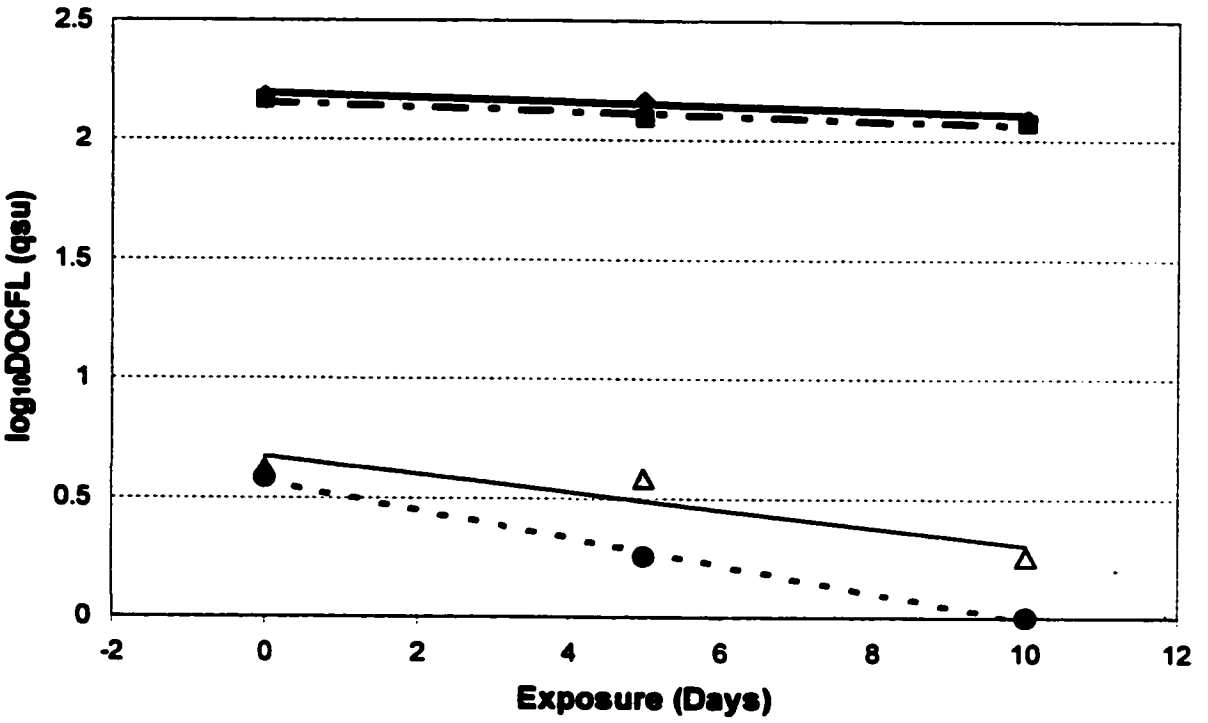

2.5
2
1.5
1
0.5
0
log10DOCFL (qsu)
-2
0
2
4
6
8
10
12
Exposure (Days)

Figure 2.7 Ln(absorbance) (m^{-1}) vs. wavelength (nm) after 0 d, 5 d and 10 d UVB exposure for Raisin River (A = 0 d, slope = -0.0170, r^2 = 0.998; B = 5 d, slope = -0.0155, r^2 = 0.998; C = 10 d, slope = -0.0153, r^2 = 0.998), Grand River (D = 0 d, slope = -0.0175, r^2 = 0.99; E = 5 d, slope = -0.0157, r^2 = 0.999; F = 10 d, slope = -0.0159, r^2 = 0.999), Lake Simcoe (G = 0 d, slope = -0.0229, r^2 = 0.993; H = 5 d, slope = -0.0218, r^2 = 0.976; I = 10 d, slope = -0.0273, r^2 = 0.934) and St. Lawrence River (J = 0 d, slope = -0.0199, r^2 = 0.981; K = 5 d, slope = -0.0200, r^2 = 0.954; L = 10 d, slope = -0.0232, r^2 = 0.759).

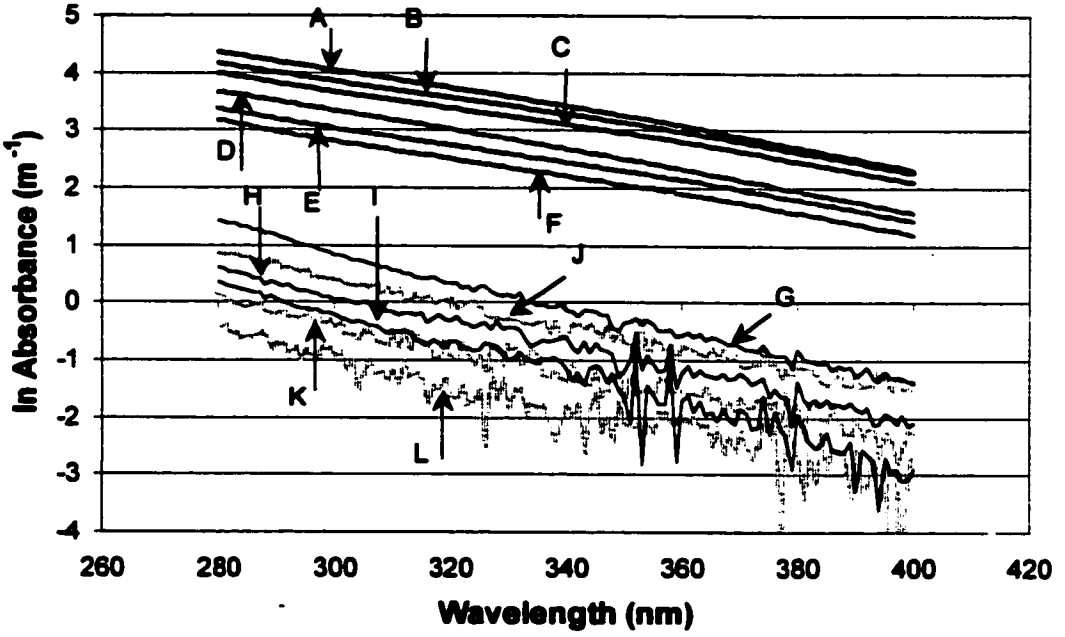

A
B
C
D
E
F
G
H
I
J
K
L
5
4
3
2
1
0
-1
-2
-3
-4
260
280
300
320
340
360
380
400
420
ln Absorbance (m-1)
Wavelength (nm)

Figure 2.8 Ln(absorbance/DOC) (m^{-1}/mg C L^{-1}) vs. wavelength (nm) after 0 d, 5 d and 10 d UVB exposure for Raisin River (RR1 - A = 0 d; B = 5 d; C = 10 d), Grand River (D = 0 d; E = 5 d; F = 10 d), Lake Simcoe (G = 0 d; H = 5 d; I = 10 d) and St. Lawrence River (J = 0 d; K = 5 d; L = 10 d).

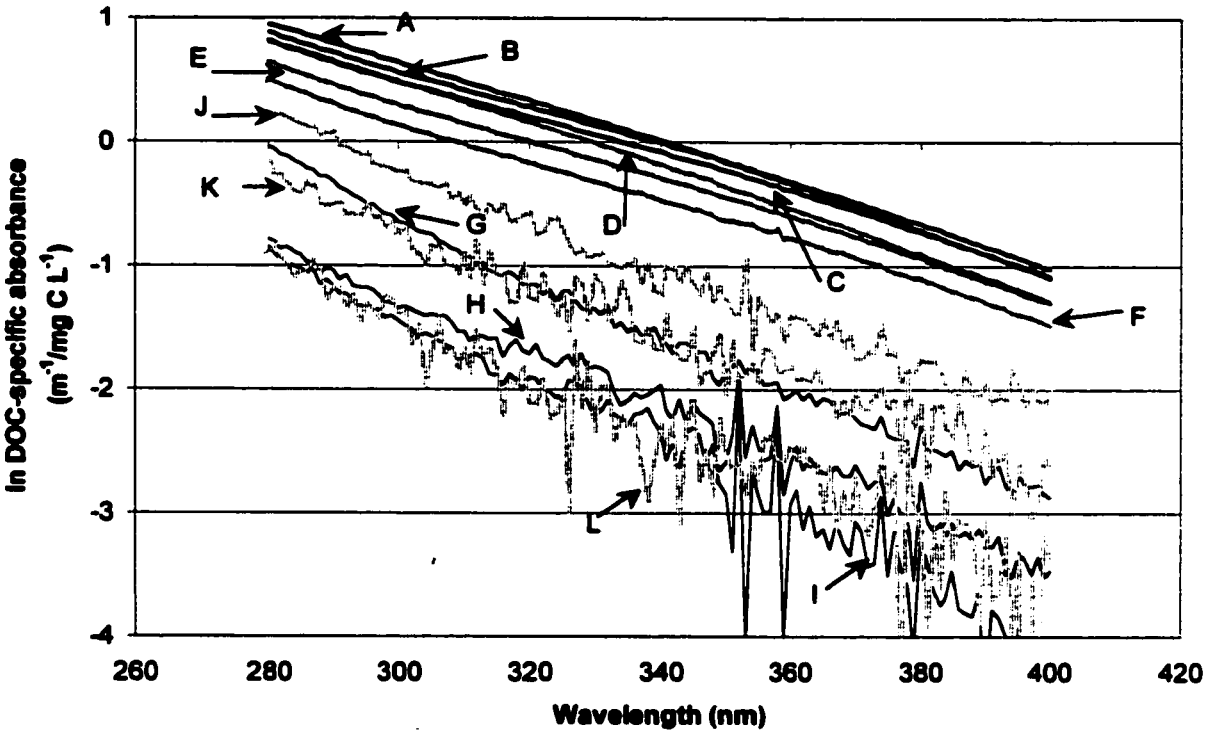
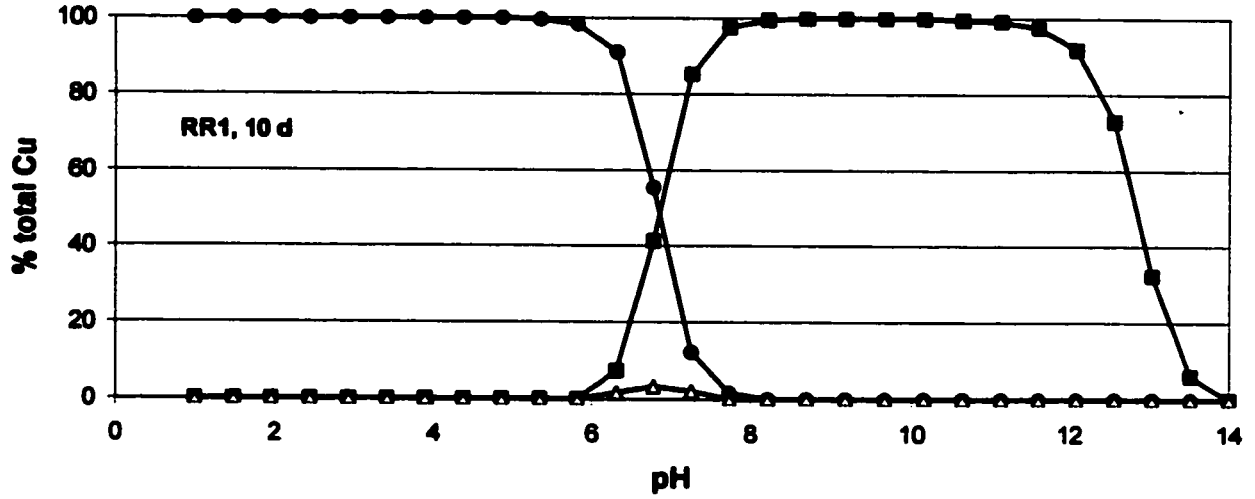
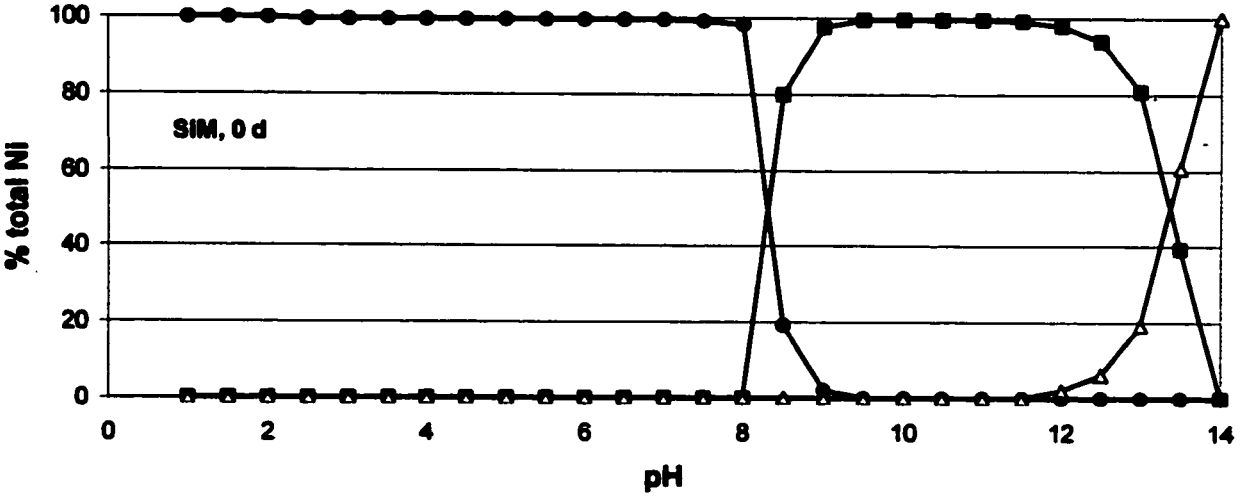

ln DOC-specific absorbance
(m-1/mg C L-1)
1
0
-1
-2
-3
-4
260
280
300
320
340
360
380
400
420
Wavelength (nm)
A
B
E
J
K
G
D
C
F
H
L
I

Figure 2.9 Speciation graphs produced by MINEQL, predicting concentrations of Cu^{2+} (filled circles), $Cu(OH)_2$ aq (filled squares) and $Cu(OH)^+$ (open triangles) in Raisin River (RR1) water irradiated 10 d and concentrations of Ni^{2+} (filled circles), $Ni(OH)_2$ (filled squares) and $Ni(OH)_3^-$ (open triangles) in non-irradiated Lake Simcoe (SIM) water at T = 25°C, I = 0.0005.


RR1, 10 d
% total Cu
pH
0
20
40
60
80
100
0
2
4
6
8
10
12
14


SIM, 0 d
% total Ni
pH
0
20
40
60
80
100
0
2
4
6
8
10
12
14

3.0 COMPARISON OF CHANGES IN METAL TOXICITY FOLLOWING EXPOSURE OF HIGH-DOC WATER TO SOLAR RADIATION AND TO UVB LAMPS

3.1 INTRODUCTION

Results of the previous experiment (Section 2) showed decreasing trends in DOC concentration, DOC fluorescence and UV absorbance for four water samples (two high-DOC brownwater samples and two low-DOC clearwater samples) irradiated under UVB lamps in the laboratory for up to 10 d. The four samples were subsequently used in algal toxicity tests. Increases in metal toxicity were observed after 10 d UVB exposure in some cases. In particular, the Raisin River sample, which contained the highest concentration of DOC, demonstrated decreasing trends in IC_{50} for four of six metals (Cu, Zn, Pb, Co), but no trends were observed for Cd and Ni.

Given the low level of UVB radiation used in the above study, and the relatively short exposure period (10 d as compared with several years' water residence time in natural water bodies), the observed change in metal toxicity results illustrates the potential importance of these processes in aquatic ecosystems.

UVB is often assumed to be the wavelength band most responsible for photochemical degradation of DOC due to the higher energy of photons in the UVB portion of the

spectrum. There is conflicting evidence in the literature regarding the relative importance of UVB as compared with UVA or PAR to change in DOC with irradiation (e.g. Granéli et al., 1998; Amyot et al., 1997; Wilson et al., 2000).

This study compares the effect of natural solar radiation on DOC to the effect of UVB from lamps when samples are exposed at an intensity approximately equal to UVB irradiance in full sun. The working hypothesis is that the degree of change in metal toxicity to *P. subcapitata* with solar exposure will correspond to the intensity of UVB in the solar radiation to which the test water is exposed. The relative impact of solar exposure vs. UVB lamps on metal toxicity was expected to vary proportionally with the relative intensity of solar UVB (compared to artificial UVB intensity) for approximately equivalent exposure time. Subsequent experiments were carried out to determine the relative effects of UVA and UVB, both from artificial sources. A secondary objective was to determine whether longer exposure times than those used in the study described in Section 2 would produce greater changes in metal toxicity in samples containing high concentrations of fresh DOC.

3.2 MATERIALS AND METHODS

3.2.1 Experiments with Newington Bog water

3.2.1.1 *Water samples*

In September, 2001, a water sample was taken from the Newington Bog in 2.2 L Teflon

bottles. Newington Bog is located approximately 20 km NW of Cornwall, Ontario (45°07' lat., 74°58' long.).

Samples were filtered through 0.45 μm Gelman mixed cellulose ester filters and stored at 4°C prior to use. As in the previous set of experiments, one set of samples was irradiated under UV lamps in the laboratory for 5 d, 9 d, 15 d and 20 d.

A second set of Newington Bog samples (after filtration) was exposed to solar radiation. Sealed 2.2 L Teflon bottles were immersed in a water bath and left in an exposed location for 20 days (September 20 to October 9, 2001, on University of Ottawa campus), with aliquots removed after 5, 12, 15 and 20 days irradiation. UVA and UVB radiation levels in the vicinity of the bottles were measured daily and at different times of the day in order to determine the range of intensity, as it fluctuated with angle of incidence and amount of cloud cover. There were approximately 5.4 d (1 d = 24 h) of clear, sunny conditions in total. The range in intensity of UVA was 1.06 (under cloud cover) to 16.14 W/m^2 (in bright sunlight). On sunny days, the average morning measurement was 6.98 W/m^2, average noon UVA was 13.00 W/m^2 and average afternoon measurement was 7.83 W/m^2. The range in intensity of UVB was 0.08 to 1.16 W/m^2, and the average morning measurement on sunny days was 0.44 W/m^2, average noon measurement was 0.96 W/m^2 and the average afternoon measurement was 0.51 W/m^2. The average daytime temperature measurement was 19.4 °C. Non-irradiated bottles were kept as controls. After irradiation, samples were stored in the dark at 4°C until used.

Some of the samples formed a reddish-brown flocculent substance in the bottom of the bottles during the irradiation process. In order to minimize interference of this substance with the results of subsequent analytical procedures, bottles were moved as little and as carefully as possible, and water was withdrawn from just below the surface with a sterile syringe.

3.2.1.2 *Algal bioassays*

Algal bioassays were performed as described in Section 2. For each of the water samples (i.e. non-irradiated controls and subsamples irradiated 5 d, 9 d, 15 d and 20 d under UVB lamps and irradiated 5 d, 12 d, 15 d and 20 d under natural sunlight), separate bioassays were conducted using sample water spiked with Cu^{2+}, Ni^{2+}, Cd^{2+} and Pb^{2+}. These metals were chosen based on the results of the previous experiment, where Cu and Pb toxicity were found to have a strong tendency to increase with UVB irradiation than Ni and Cd. In each case, a working solution was initially made by dissolving dry metal nitrate salts (purchased from Aldrich) in sample water to obtain the highest metal concentration to be used in the bioassay (maximum concentrations are listed in Table 3.1). Calculations of metal content were based on percent metal by weight as indicated on the certificate of analysis for each metal. Nitrates were used rather than sulfates because N is a relatively weak complexing agent for metals. Concentrations of nitrate added were negligible compared with the concentration of nitrate in the nutrient spike prepared in accordance with the Environment Canada protocol. The highest concentration of metal nitrate used

(1000 μg/L Ni) would provide 477 μg/L of N, a relatively small amount when compared with the 15.94 mg/L $NaNO_3$ (2630 μg/L N) from the nutrient spike (see Table 2.2).

Working solutions were prepared three days prior to the initiation of the bioassays. Use of the dry salts was found to be preferable to the metal nitrate solutions, as the salts did not significantly change the pH of the sample, therefore pH adjustment was not required. Working solutions were shaken vigorously and allowed to equilibrate at 4°C until the test date.

A calibration curve was established for chlorophyll-a fluorescence (measured using the microplate spectrofluorometer) in non-irradiated Newington Bog water by the methods described in Section 2. The calibration curve was linear ($r^2 = 0.99$).

3.2.1.3 *DOC characterization*

DOC concentration, DOC fluorescence and absorbance (200-800 nm) were measured as described in Section 2. Absorbance (cm^{-1}) was plotted against wavelength for each time interval (e.g. 0 d, 5 d, 9 d, 15 d and 20 d for UVB-irradiated water). In order to examine the changes in UV absorbance in finer detail than can be easily observed from untransformed absorbance scans, cumulative UV absorbance loss (200 - 400 nm) was then plotted vs. wavelength for each time interval. In other words, the 5 d , 9 d, 15 d and 20 d absorbance spectra were each subtracted from the 0 d spectrum for UVB-irradiated bog

water, and the 5 d , 12 d, 15 d and 20 d absorbance spectra were each subtracted from the 0 d spectrum for solar-irradiated water.

Nitrate is known to absorb strongly at ~ 200 nm. $NaNO_3$ solutions containing 0.5, 1, 2.5 and 5 mg/L NO_3^- were prepared in deionized water and scanned from 200 to 800 nm in the spectrophotometer. A plot of absorbance vs. NO_3^- concentration was linear for 200 nm, 206 nm and 215 nm ($r^2 = 0.9997$, 0.9993 and 0.9997, respectively). The nitrate absorbance curves were then used to estimate absorbance of the nitrate concentrations measured in the unirradiated and irradiated Newington Bog samples (0 d, 9 d and 20 d UVB; 0 d, 12 d, 20 d solar).

3.2.1.4 *Analysis of anions and cations*

Anions and cations were measured by ion chromatography for bog water exposed to 0 d, 9 d and 20 d UVB, 12 d and 20 d solar radiation.

3.2.2 Experiments with Raisin River water

Based on the results from the above-described experiments on Newington Bog water, particularly DOCFL trends, some additional experiments were run with a new Raisin River (RR2) sample. The sample was taken from under ice in January, 2002. The purpose of these experiments was to verify trends observed in the Newington Bog experiments by

irradiating high-DOC water from the Raisin River with UVA and UVB lamps in the laboratory and then measuring DOC, DOCFL and absorbance.

The RR2 sample was split and one set of samples was irradiated with UVB as described above for Newington Bog water. A second set was irradiated using UVA lamps. Bottles were placed at a distance from the lamps where they would receive 10.00 W/m^2 UVA at the bottle surface, in order to approximate the irradiance UVA in solar radiation, as measured during the Newington Bog experiment. Spectral irradiance was also measured in bottles filled with RR2 sample water, following the method described in Section 2. Two bottles of non-irradiated sample were stored in the dark at 4°C until use. A 50 mL sample was removed from each of the irradiated bottles after 5 d, 10 d, and 15 d irradiation, and the remainder of each bottle was kept as the 20 d subsample. All samples were stored in the dark at 4°C until use.

After irradiation, all RR2 samples were analyzed for DOC concentration, DOCFL and absorbance, following the methods outlined in Section 2, and absorbance loss curves were generated as described for the Newington Bog experiments. Bioassays were performed on samples irradiated 0 d and 20 d (UVA and UVB). The bioassays were conducted in the same manner as the ones performed on Newington Bog water, except that no bioassays were conducted on the interim irradiation treatments due to an insufficient quantity of sample.

3.3 RESULTS

3.3.1 Newington Bog Experiments

3.3.1.1 *Algal bioassays*

Figure 3.1 presents the results of bioassays using both solar and UVB irradiated samples. At the end of 20 d UVB irradiation, IC_{50} was significantly reduced for all four metals: Pb - 64%; Cu - 63%; Ni - 35% and Cd - 40%. In the solar treatments, after 20 d exposure IC_{50} also decreased for all four metals (Pb - 58%; Cu - 49%; Ni - 18% and Cd - 23%), but only the changes in IC_{50} for Pb and Cu were statistically significant. All values of IC_{50} and their 95% confidence limits are listed in Appendix A.

3.3.1.2 *DOC concentration*

IC_{50} values decreased by as much as 64%, but DOC declined much less. DOC concentrations were reduced in both samples, but less in the solar-irradiated samples (11% after 20 d) than in the UVB-irradiated samples (20% after 20 d). Figure 3.2 presents $\log_{10}$DOC for all Newington Bog samples. The slopes of $\log_{10}$DOC vs. exposure time for UVB and solar-irradiated samples differed significantly ($p < 0.0001$). Linear regression on both data sets produced a slope of -0.0023 ($r^2 = 0.89$, $n = 20$) for the solar-irradiated sample and -0.005 ($r^2 = 0.97$, $n = 13$) for the UVB sample. The rate constants for DOC

loss were computed as:

$k_{sun} = 2.303\ (-0.0023) = -0.00530\ d^{-1} \therefore d[DOC]/dt = -0.00530[DOC]\ (mg\ C\ L^{-1}\ d^{-1})$

and

$k_{UVB} = 2.303\ (-0.0050) = -0.01151\ d^{-1} \therefore d[DOC]/dt = -0.01151[DOC]\ (mg\ C\ L^{-1}\ d^{-1})$

If DOC degradation continued at the rate observed, and fresh DOC replaced that being degraded so that a steady state level of DOC was maintained, the DOC turnover time would be 1/0.00530 ≈ 189 d for the solar experiment, and 1/0.01151 ≈ 87 d for the UVB experiment.

DOC concentration was reduced by UVB in Newington Bog water at approximately half the rate of reduction in Raisin River (RR1), Grand River and Lake Simcoe water. The difference is likely due to self-shading in the darker bog water.

Some reddish-brown precipitate was visible in the solar treated bottles, and much more appeared in the UVB-irradiated bottles, particularly after > 9 d irradiation. No analysis was performed on this substance.

3.3.1.3 *DOC fluorescence*

In the Newington Bog water, DOCFL values gradually dropped from an initial value of 164 qsu to 110 qsu (33%) after 20 d solar exposure. Surprisingly, this decrease is larger than the corresponding decrease in DOCFL after exposure to UVB lamps (164 to 136 qsu,

a 17% drop), even when solar values are not normalized for exposure time. Figure 3.3 is a plot of $\log_{10}$DOCFL vs. exposure time for both UVB and solar-irradiated samples. The slopes of $\log_{10}$DOCFL vs. exposure time for UVB and solar-irradiated samples differed significantly ($p < 0.0001$), but in the opposite direction from $\log_{10}$DOC, i.e. linear regression produced a slope of -0.0080 ($r^2 = 0.92$, n = 13) for the solar-irradiated sample and -0.0037 ($r^2 = 0.86$, n = 20) for the UVB sample. The rate constants for DOCFL reduction in these samples were computed as:

$$k_{sun} = 2.303\ (-0.0080) = -0.0184\ d^{-1} \therefore d[DOCFL]/dt = -0.0184[DOCFL]\ (qsu\ L^{-1}\ d^{-1})$$

and

$$k_{UVB} = 2.303\ (-0.0037) = -0.0085\ d^{-1} \therefore d[DOCFL]/dt = -0.0085[DOCFL]\ (qsu\ L^{-1}\ d^{-1})$$

DOCFL turnover time was calculated to be ~ 54 d for the solar treatment, and ~ 117 d for UVB.

DOCFL was reduced by UVB in Newington Bog water at approximately 42% of the rate of reduction in Raisin River (RR1) and Grand River water.

3.3.1.4 *UV absorbance*

Absorbance scans normalized for DOC concentration for water irradiated with UVB for 0 d, 9 d and 20 d, and water exposed to solar radiation for 0 d, 12 d and 20 d appear in Figure 3.4. Under both exposure regimes, UV absorbance decreases with increased exposure time, and these changes are more significant for shorter wavelengths, in

accordance with results from Section 2. Less change in UV absorbance was observed in the solar-irradiated samples than in UVB samples.

In Newington Bog water, absorbance at 200 nm (a_{200}) decreased by 0.32 cm^{-1} after 9 d UVB irradiation (before adjustment for DOC concentration). During the same interval, nitrate concentration dropped from 5.35 to 0.25 mg L^{-1}, a loss of 5.10 mg L^{-1} NO_3^-. This concentration of nitrate in deionized water would produce $a_{200} \sim 0.7$ cm^{-1}, therefore it could be expected that at least as much absorbance would be lost from water samples after 9 d UVB irradiation. The observed absorbance loss, however, was less than half this value before taking into account loss of absorbance attributable to DOC removal or alteration. The nitrate concentration measured in the bog water after 20 d irradiation was 0.47 mg L^{-1}, so no further loss of NO_3^- occurred between 9 and 20 d (measurements indicate a slight increase). Since a_{200} loss between 9 and 20 d is equal to a_{200} loss between 0 and 9 d (0.32 cm^{-1} before DOC adjustment), the influence of nitrate concentration on absorbance in Newington Bog water was minimal. In other words, the same loss in absorbance was observed in 11 days after the NO_3^- was largely depleted as in the first 9 days of irradiation. Verification of the influence of an addition of 5 mg NO_3^- L^{-1} on samples from Newington Bog, RR2, Grand River, Lake Simcoe and St. Lawrence River showed that absorbance values for nitrate and DOC (200 - 240 nm) are not additive, and that nitrate absorbs more strongly in clearer, lower-DOC water (see Figure 3.5).

In the solar-irradiated samples, nitrate concentration was reduced by 4.88 mg L^{-1} between 0 and 12 d, which would once again be expected to produce a loss in a_{200} of ~ 0.7 cm^{-1}. The observed loss between 0 and 12 d was 0.26 cm^{-1}, again less than half the predicted value before taking absorbance loss from DOC removal and/or alteration into account. The influence of nitrate on the absorbance curves for Newington Bog water appears to be negligible. Between 12 d and 20 d, absorbance loss in the solar-irradiated samples was comparatively trivial (0.02 cm^{-1}).

Nitrite concentrations increased from an initial value of 0.04 to 5.51 mg NO_2 L^{-1} in the first 9 d of UVB exposure and remained steady (5.51 mg NO_2 L^{-1}) between 9 d and 20 d UVB. In the solar treatment, nitrite concentrations increased slightly from 0.04 to 0.05 and 0.21 mg NO_2 L^{-1} after 12 d and 20 d exposure, respectively.

Figure 3.6 presents cumulative absorbance loss curves for UVB and solar-irradiated samples, normalized for DOC concentration. (No adjustments were made for nitrate absorbance to absorbance loss curves in Figure 3.6 because absorbance of an additional 5 mg NO_3^- L^{-1} cannot be assumed to produce the same changes in absorbance as the initial nitrate concentration in the sample.) Loss of absorbance per mg C L^{-1} at all wavelengths is greater after 20 d solar irradiation than 20 d UVB, even without adjusting for exposure time. The shapes of the absorbance loss curves are different for the two treatments. Although there is no discernible peak in absorbance loss between 0 d and 20 d with solar irradiation, the curve features a broad shoulder between 260 and 350 nm. With increased

exposure time, DOC-specific absorbance at all wavelengths increased steadily. Absorbance loss between 0 d and 5 d for the UVB treatment features a narrower peak centred around ~ 277 nm and less absorbance loss at wavelengths > 300. As time progressed, the peak in absorbance loss centred around 277 nm shifted toward the shorter wavelengths. All of the absorbance loss per unit DOC occurred between 0 and 9 d with UVB exposure. After 15 d, the solar-irradiated samples began to show a slight reduction in absorbance loss at wavelengths < 220 nm.

3.3.2 Experiments on Raisin River (RR2) water

3.3.2.1 *Spectral Irradiance*

Irradiance from UVB lamps integrated over 280-320 nm was reduced from 1.38 W/m^2 in deionized water to 0.01 W/m^2 in the same Teflon bottle filled with RR2 sample water. Similarly, irradiance from UVA lamps integrated over 320-400 nm was reduced from 7.07 to 0.63 W/m^2.

3.3.2.2 *Algal bioassays*

Results of IC_{50} calculations for the RR2 bioassays are shown in Figure 3.7. Significant decreases in IC_{50} were found only after 20 d UVA for Cu, and after 20 d UVB for Pb. IC_{50} values for all metals were lower in the RR2 sample than in Newington Bog water, except for Pb irradiated 20 d, where the Newington Bog IC_{50}s for both UVB and solar treatments dropped below the 20 d UVB and UVA values, respectively, in RR2.

Cu IC_{50} increased between 15 d and 20 d on the roof. The bottle that was irradiated for 20 d, however, was later found (by light microscopy) to contain some algae that escaped the filtering process. Since thiols produced by some phytoplankton have been shown to bind Cu very strongly (Tang et al., 2001), this could explain the increase in IC_{50} in the 20 d sunlight-irradiated water.

3.3.2.3 *DOC concentration*

DOC concentration decreased linearly from 20.7 to 15.7 mg C/L (24%) in RR2 irradiated with 20 d UVA and from 20.7 to 16.5 (20%) with 20 d UVB. The difference between the effects of UVA and UVB on DOC concentration was found to be statistically insignificant (ANOVA $p = 0.1959$). Log_{10}DOC vs. UV exposure is plotted in Figure 3.8. Based on the average of the two slopes, the rate constant for DOC loss was calculated to be -0.01244. Some reddish-brown residue formed in the bottles irradiated for longer time periods, particularly with UVB, as observed in the previous experiments.

3.3.2.4 *DOC fluorescence*

The UVB-treated RR2 water lost 7.7% DOCFL (149.3 to 137.8 qsu) following 20 d exposure, and the reduction in DOCFL was linear from 0 to 20 d. DOCFL in RR2 water exhibited a diphasic response to UVA treatment, dropping from 149.3 to 72.4 qsu (51.5%) between 0 d and 5 d UVA. Figure 3.9 is a plot of DOCFL vs. exposure time. After the initial 5 d period, the difference in the rate of decrease of DOCFL under UVA treatment between 5 and 20 d was statistically insignificant from the corresponding rate for the UVB

treatment (ANCOVA p = 0.1451). Final DOCFL values after 20 d irradiation were 37.8 qsu and 97.4 qsu for UVA and UVB treatments, respectively.

3.3.2.5 *UV absorbance*

Absorbance spectra for 0 d, 20 d UVA and 20 d UVB appear in Figure 3.10 and absorbance loss curves (normalized for DOC) in Figure 3.11. Loss of absorbance occurred with both UVA and UVB treatments, and more loss was observed in the UV region of the spectrum than in wavelengths > 400 nm. The DOC-specific absorbance loss curve after 5 d UVA features a broad peak with a maximum at ~ 301 nm. The corresponding peak for UVB has a maximum at ~ 279 nm and is narrower than the UVA peak. The maxima are not of the same intensity: 0.05146 cm^{-1} for UVB and 0.03791 cm^{-1} for UVA. After 20 d exposure, absorbance loss is greater with UVA than UVB at all wavelengths before normalizing for DOC concentration, but after normalizing, absorbance loss is greater with UVB than UVA from ~ 255 - 300 nm, and the reverse is true from 300 to >400 nm.

3.4 DISCUSSION

3.4.1 20 d UVB vs. 10 d UVB exposure

Algal bioassays on UVB-irradiated Newington Bog water (initially containing 35.1 mg C/L) produced evidence to support the hypothesis that exposure of high-DOC water to UVB radiation over a 20 d period would result in significant changes in toxicity of selected metals, and that the change in toxicity would be more significant with 20 d than 10 d

exposure to UVB. IC_{50} for all four metals tested underwent a statistically significant decrease after 20 d UVB exposure of the bog water. By contrast, the results of Section 2 show that of the same four metals tested in Raisin River (RR1) water, only Cu and Pb underwent a significant decrease in IC_{50} after 10 d, and Ni and Cd IC_{50}s remained virtually unchanged (< 3% change).

Although decreases in IC_{50} were also observed in all four metals (Pb, 43%, Cu, 28%; Ni, 20% and Cd, 11%) tested in RR2 water (initially containing 20.7 mg C/L) exposed to 20 d UVB, these effects were less intense than the corresponding decreases in IC_{50} in the bog water. Only Pb produced a statistically significant decrease. Increasing UVB exposure time from 10 d to 20 d did not produce similar results in these two different high-DOC samples, leading to the conclusion that the degree of change in metal toxicity does not depend solely on UVB exposure time, but also on the initial DOC concentration of the water sample in question.

DOC concentration, however, does not predict the change in toxicity of all metals equally for a given UVB exposure time. In both Newington Bog and RR2 water, decreases in IC_{50} were most important for Pb, followed by Cu > Ni > Cd. IC_{50} for all four metals tested in Newington Bog water, and Cu and Pb in RR2 water outstripped the 20% change in DOC concentration produced by 20 d UVB irradiation for both samples. In the bog water, for example, IC_{50} for Pb decreased by 63%, over three times the percent decrease in DOC. Clearly some components of DOC are being affected by UVB more strongly than others.

Different metals are known to bind to different sites in DOC (Mandal et al., 2002). The varying degrees of change in toxicity of the metals tested in this study suggest that some organic metal-binding ligands are more quickly removed or altered than others when exposed to UVB radiation. The DOC remaining after irradiation appears to be qualitatively different from the unirradiated DOC, and this difference is reflected both in the lower capacity of the remaining DOC to mitigate toxicity of several different metals, and in the varying degrees of change in toxicity of individual metals.

A comparison of results from UVB exposure in Newington Bog and Raisin River (RR1) samples shows that the decline in IC_{50} of Cu and Pb outpaced the decrease in DOC concentration after 5 d or less. These observations have quite serious implications for short-term changes of Pb and Cu speciation in well-mixed water, since, according to Whitehead et al. (2000), faster mixing is associated with larger DOC absorbance losses and faster loss rates due to photochemical reactions. In slow-moving systems, absorbance loss (and therefore loss of protective characteristics) is confined to a thin layer near the surface. In faster systems, up to three times the amount of fresh DOC passes through the UV photoactive layer in the same time period as in slow systems. Self-shading, therefore, becomes less important and photoreaction rates are faster, implying that protection from metal toxicity would be eroded more quickly in fast-moving systems than in slow ones. In the DOC-specific absorbance loss curve for 20 d UVB, the peak centred around 279 nm indicates preferential removal of specific compounds. Although the precise nature of these compounds cannot be determined from this study, it is probable that they are aromatic and

highly substituted with polar, oxygen-containing functional groups. In examining changes to UV absorbance of DOC with chlorination, Korshin et al. (1997) use benzene, the simplest of aromatic molecules, as a model. UV absorbance by aromatic compounds is summarized in Korshin et al. (1997). Briefly, benzene exhibits peaks in absorbance at 180 nm, 203 nm and 253 nm, corresponding to the energy required to promote electrons from the ground state ($^1A_{1g}$) to the π-antibonding orbitals corresponding to energy levels $^1E_{2u}$, $^1B_{1u}$, $^1B_{2u}$. These three spectral bands have been denoted LE (local-excitation band), Bz (benzenoid band) and ET (electron transfer band), respectively. Progressing from LE to Bz to ET, each successive band is less intense and peaks at a longer wavelength than the previous one. The Korshin et al. (1997) study is based on the assumption that each aromatic chromophore in DOC possesses a similar set of LE, Bz and ET bands, although the peaks may fall at different wavelengths from the corresponding bands in benzene. In natural water, UV absorbance scans reflect composite LE, Bz and ET bands to which all of the aromatic compounds in the DOC contribute. Despite some overlapping, these bands are distinct from one another and can be identified from UV absorbance spectra. The degree of substitution of the aromatic rings with polar, oxygen-containing functional groups ("activation" of aromatic rings) likely changes absorbance intensity in each of the bands, but not the corresponding peak wavelength, therefore the LE, Bz and ET bands in DOC can be expected to peak at $\lambda < \sim 190$ nm, $190 \text{ nm} < \lambda < 230$ nm and $240 < \lambda < 400$ nm, respectively. The ET band changes more in response to destruction of polar, oxygen-containing functional groups than either the Bz or LE bands, and changes very little with concentrations of non-polar or aliphatic functional groups.

The peak in absorbance loss at 279 nm falls within the limits of the ET band. Preferential loss in this band can be interpreted as preferential loss of polar, oxygen-containing functional groups. Some such groups (e.g. COOH or OH) are also known to be metal-binding sites.

3.4.2 Solar radiation vs. UVB lamps

A comparison of the effects of UVB lamps and solar radiation on Newington Bog water must take into consideration the characteristics of the solar treatment. During the irradiation period (late September to early October, in Ottawa), there were only ~ 12 h of daylight each day. Further, intensity of all wavelengths varied with cloud cover and time of day (as described in the Materials and Methods section), and both the average and the maximum UVB intensity measured during solar irradiation was considerably lower than the UVB intensity of the lamps.

In order to equalize exposure times to the best approximation possible, this section compares results from incubation of Newington Bog water for 20 d outdoors with those from 5 d UVB exposure in the laboratory. Given the relatively low levels of UVB measured in the solar radiation, the outcome can be considered a conservative estimate of the relative impact of solar exposure vs. UVB on metal toxicity and DOC.

The comparison revealed that solar radiation had a greater impact than UVB on toxicity of Cu (38% UVB, 49% sun) and Pb (50% UVB, 58% sun), an equal impact on Ni toxicity

(18% for both UVB and sun) and a smaller impact on Cd toxicity (31% UVB, 23% sun). Binding sites for specific metals appear to be affected to greater or lesser degrees by different wavelength bands.

The solar treatment also reduced DOC concentration (11%) more than UVB (4.6%), and DOCFL decreased sizeably more (33% sun; 8% UVB). Solar irradiation reduced DOC-specific UV absorbance to a greater extent than UVB, particularly at longer wavelengths.

This comparison gives evidence that UVB is not the only waveband in solar radiation that can affect the quantity and quality of DOC in a water body. At the intensities of solar irradiance associated with clear, sunny conditions, UVA alone or in combination with UVB and/or PAR affects DOC concentration, UV absorbance, and toxicity of Cu and Pb more intensely than UVB alone. The much greater irradiance of UVA (and possibly PAR) makes its contribution to the removal and/or alteration of DOC at least as important as the influence of higher energy UVB.

In response to both the UVB and the solar radiation treatments, nitrate levels in Newington Bog water decreased by over 80%, and this change was observed after 9 d and 12 d for UVB and solar treatments, respectively. The UVB treatment also resulted in an increase of nitrite concentration that was approximately equivalent to the loss of NO_3^-. Only a slight increase in nitrite concentration was observed in response to the solar exposure.

NO_3^- is known to be reduced by exposure to solar radiation in the UVB wavelength band (~ 302 nm) by the following reactions:

$$NO_3^- + h\nu \rightarrow NO_3^{-*} \rightarrow NO_2^- + O(^3P)$$

followed by removal of atomic oxygen, $O(^3P)$, by reactions with NO_2^- or by decomposition to $^{\bullet}OH$ (summarized in Zepp et al., 1987). NO_2^- is, in turn, photolyzed by solar radiation to produce $^{\bullet}OH$, of which mean quantum yield is greatest at wavelengths ~ 337 nm and ~ 300 nm (Zafiriou and Bonneau, 1987). Greater solar irradiance at ~ 337 nm than at ~ 300 nm might explain the observed difference in nitrite levels between the samples exposed to UVB lamps and solar radiation, since nitrite is photolyzed at both wavelengths.

3.4.3 UVA vs. UVB exposure

UVA was found to be of at least the same importance in reducing DOC concentration as UVB after 20 d exposure, confirming that UVB should not be considered the only wavelength band in solar radiation to remove DOC from fresh waters. Although UVA has less energy per photon than UVB, UVA irradiance reaching the earth's surface is an order of magnitude greater than that of UVB. Furthermore, in this study, UVA irradiance was only ~ 4x UVB irradiance, therefore these results can be considered a conservative estimate of the influence of UVA on DOC concentrations, as compared with UVB. UVA is also absorbed and attenuated much less than UVB in natural waters. The vertical attenuation coefficient, K_d, has been calculated as $0.415(DOC)^{1.86}$ m^{-1} for UVB and $0.299(DOC)^{1.53}$ m^{-1} for UVA (Lean, 1998 a). With depth in a water column, UVA would be more important than UVB with respect to DOC photobleaching and alteration.

In RR2 samples, algal bioassay results showed that 20 d UVA and UVB treatments produced different effects on the four metals tested. Statistically significant decreases in IC_{50} were observed only for Cu (47%) with UVA and Pb (43%) with UVB. These observations are consistent with the concept of different ligands in DOC being affected by different wavelength bands. That Cu toxicity is more strongly affected by UVA than UVB is consistent with results of the solar experiments described above, but IC_{50} for Pb decreased only 10% with 20 d UVA, therefore Pb cannot be said to be affected more strongly by UVA alone than by UVB alone.

DOCFL loss in RR2 water with 20 d UVA was much greater than with 20 d UVB, indicating that UVA removes fluorescent compounds (excitation 365 nm; emission 437 nm) much faster than UVB does. This is the most obvious explanation for the pronounced decrease in DOCFL in the sunlight experiment, since solar radiation contains much more UVA (and photosynthetically active radiation - PAR) than UVB. The contrast between changes to DOCFL in RR2 water irradiated with UVA and UVB and the corresponding bioassay results shows that DOCFL does not predict metal toxicity. (If it did, IC_{50}'s would have been lower after UVA treatment.)

Absorbance data from the RR2 experiment indicate that UVA and UVB preferentially affect different types of DOC molecules, especially in the first 5 d of exposure. The DOC-specific absorbance loss curves (see Figure 3.11) show that after 5 d, UVA removes more kinds of molecules than does UVB, and that it has a greater effect on molecules that absorb

at longer wavelengths. The ratio of a_{250}:a_{340} is often used as an estimate of the proportion of smaller to larger organic molecules in DOC (Strome & Miller, 1978). If larger molecules absorb at longer wavelengths, then these observations imply that UVA decreases absorbance of larger molecules and of a broader range of molecules than UVB.

The maximum DOC-specific loss of absorbance between 250 and 400 nm from the RR2 sample after 5 d was lower following UVA than UVB treatment. If these peaks represent loss in absorbance by aromatic groups, the more dramatic drop in DOCFL after 5 d UVA treatment would seem contrary to the absorbance results, because fluorescence is also representative of aromaticity. However, DOCFL is measured as emission at 437 nm after excitation at 365 nm, and there is a high positive correlation between intensity of DOC absorbance at the excitation wavelength and fluorescence at the emission wavelength (Nieke et al., 1997). It is not surprising, therefore, that the UVA treatment showed much greater loss of DOCFL as measured in this experiment. UVA decreases absorbance in more molecules that absorb at the higher end of the UV region than does UVB, and after 5 d irradiation of RR2 water, UVA reduced absorbance by 0.02118 cm^{-1}, 64.5 times more than did UVB at the same wavelength (0.00033 cm^{-1}).

In both UVA and UVB treatments, the ET peak (inflection point on the absorbance spectrum) shifted in the direction of shorter wavelength. This is not surprising given that both UVA and UVB induced maximum loss of absorbance at longer wavelengths than the ET peak, and these maxima, although diminished, moved in the direction of shorter

wavelengths as irradiation time progressed. If larger aromatic molecules absorb at longer wavelengths, this shift implies fragmentation of larger aromatic molecules into smaller ones that retain some degree of aromaticity. The conversion of high molecular weight (HMW) DOC compounds to low molecular weight (LMW) molecules (most of which have MW < 100) is well documented in the literature. Several studies have found that the end products of this process include formaldehyde, acetaldehyde, acetone, glyoxal, methylglyoxal, glyoxylate, pyruvate, CO and others after irradiation (e.g. Mopper et al., 1991; Kieber et al., 1990; Kieber et al., 1989). No experiments were performed in this study, however, to verify change in molecular weight following UV exposure, and no studies have yet examined the quantities of LMW molecules produced as compared with decreases in DOC concentration. The conversion rate of DOC to identifiable photoproducts has, however, been determined to be less than 20% of the bleaching rate of DOC (Miller and Zepp, 1995 in Moran and Zepp, 1997), therefore it is unlikely that this process accounts for more than a small portion of the decrease in DOC with UV irradiation.

UVB had a stronger negative impact on IC_{50}s of three of the four metals tested (Pb, Ni, Cd) than UVA. UVB also produced greater loss of DOC-specific absorbance between 255 and 300 nm after 20 d. These results imply that Cu binding sites absorb at longer wavelengths than the other three metals. If larger molecules absorb at longer wavelengths, these results can be interpreted to mean that Cu binds to aromatic compounds of higher molecular weight than Pb, Ni or Cd.

The results of this study are comparable to other examples in the literature where the relative effects of UVB, UVA and PAR have been examined. For example, Granéli et al. (1998) found that UVB explained only a minor part (17%) of photooxidative DIC production, compared with UVA (at least 39%) and PAR (up to 44%) in Swedish and Brazilian lakes ranging in DOC content from 2.9 to 41.8 mg C L^{-1}, even though UVB intensity in Brazil was approximately three times the intensity in Sweden. Another study (Amyot et al., 1997) examined dissolved gaseous mercury production (DGM) from lake waters by exposure to solar radiation and found that in Plastic Lake (2.2 mg C L^{-1}), DGM production was 37% lower when the UVB waveband was removed, and 77% lower when both UVA and UVB wavebands were blocked, but no such trends were observed in samples from Ranger and Fawn lakes (5.0 and 8.7 mg C L^{-1}, respectively). H_2O_2 formation from photochemical reactions between DOC and UVA radiation has also been shown to be 2.5-3 times that formed in water irradiated with UVB lamps (Wilson et al., 2000).

On the other hand, Kieber et al. (1990) attribute photoproduction of LMW carbonyl compounds and photobleaching solely to UVB, as it is correlated with bleaching of DOC absorbance in this wavelength band. This study shows, however, that irradiation with UVA also reduces absorbance in the UVB band.

In spite of equivalent or greater decreases in DOC concentration, DOCFL and absorbance

at all wavelengths, UVA does not appear to increase toxicity of the tested metals to the same extent as UVB, with the exception of Cu.

These results may have implications for phytoplankton community structures. *P. subcapitata* has been shown to be highly sensitive to metal toxicity, but other species of green algae, e.g. *Scenedesmus quadricauda, Scenedesmus subspicatus* and *Chlamydomonas reinhardtii* have comparable metal tolerance levels (Rojickova-Padrtova and Marsalek, 1999). The limiting levels of phosphates found in natural water have been shown to reduce IC_{50} of Cd in *P. subcapitata* by a factor of 10.6 compared with nutrient levels used in standard laboratory bioassays (Lin et al., 1996). Nutrient limitation may intensify the effects of UV radiation on metal toxicity to algae in natural waters, and in the long term may cause reductions in phytoplankton concentrations or changes in species composition.

The intensities of UVA and PAR at the earth's surface do not increase with ozone thinning. If these wavelength bands, alone or in combination, are at least as important to the destruction and/or alteration of DOC as is UVB, then ozone thinning may not have as much of an impact on DOC concentration and UV absorbance as previously thought. On the other hand, degradation of DOC by UVA and UVB implies that it is not safe to assume DOC will bind metals to the extent predicted by initial concentrations. As water moves downstream from peatlands through streams and rivers to lakes and oceans, DOC is progressively degraded by UV and diluted with older water. Further, although UVB had

more of an impact on metal toxicity than UVA in this study, UVA and PAR penetrate further into the water column than UVB, and may initiate the process of DOC degradation at greater depths, an especially important consideration in well-mixed systems.

3.5 CONCLUSIONS

UV irradiation at the intensities found in solar radiation was found to reduce DOC concentration, UV absorbance, DOCFL, and increase metal toxicity in natural fresh waters.

Changes in metal toxicity after UV exposure, however, are not uniform for all metals. Increases in toxicity were found to outpace decreases in DOC concentration, particularly for Cu and Pb. The degree of change in toxicity with UV irradiation may depend not only on UV exposure time, but also on the metal being tested, the initial DOC concentration of the water and the wavelength band of irradiation.

At approximately the intensity of natural solar radiation, UVA decreases DOC concentration by at least the same amount as UVB. It also has a much greater impact than UVB on DOCFL (ex 365 nm; em 437 nm), due to the fact that absorbance at 365 nm is reduced faster by UVA than UVB. UVA targets more specifically molecules that absorb at longer wavelengths than UVB, and therefore may affect different metal binding sites in DOC. Both UVA and UVB preferentially remove DOC molecules containing aromatic

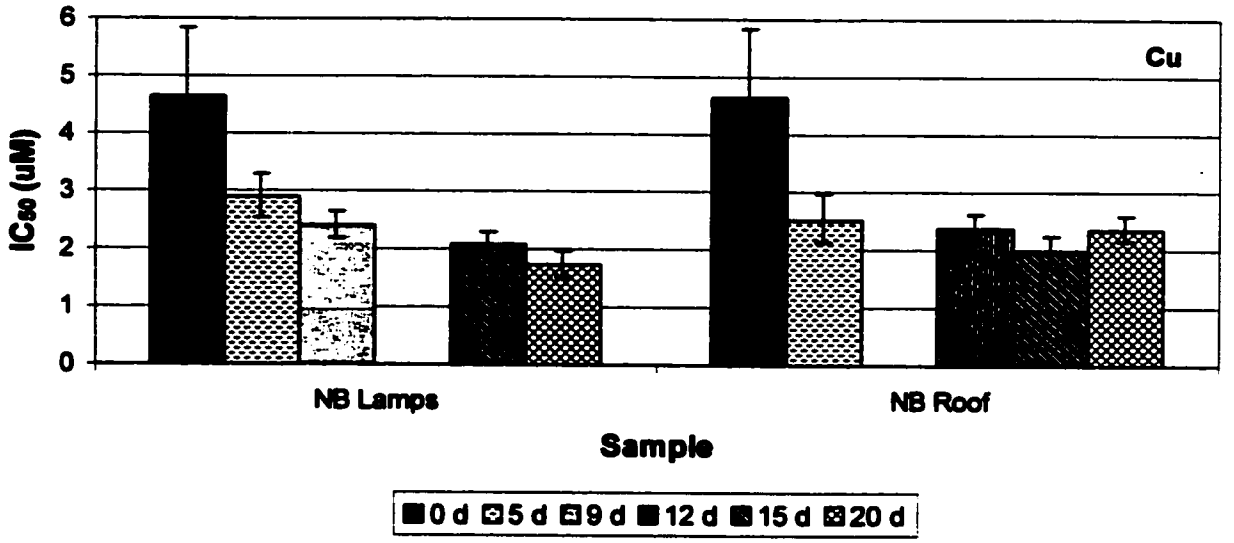
rings, but UVB removes a narrower range of groups that absorb at shorter wavelengths than does UVA.

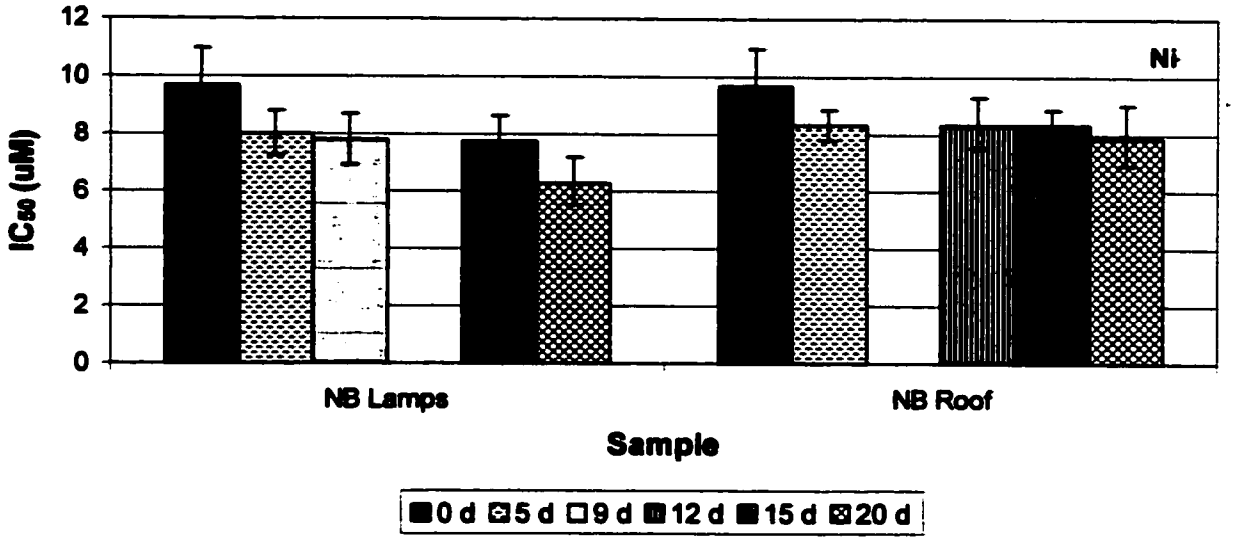
These findings may have implications for phytoplankton concentrations and community compositions in natural freshwater systems, particularly if they are well-mixed. As well, if UVA is at least as important to the alteration and/or degradation of chromophoric DOC than UVB, increased UVB as a result of ozone depletion may not have as much of an impact on DOC degradation as previously thought. However, UVB may have a more serious impact than UVA on toxicity of metals such as Pb, Ni and Cd.

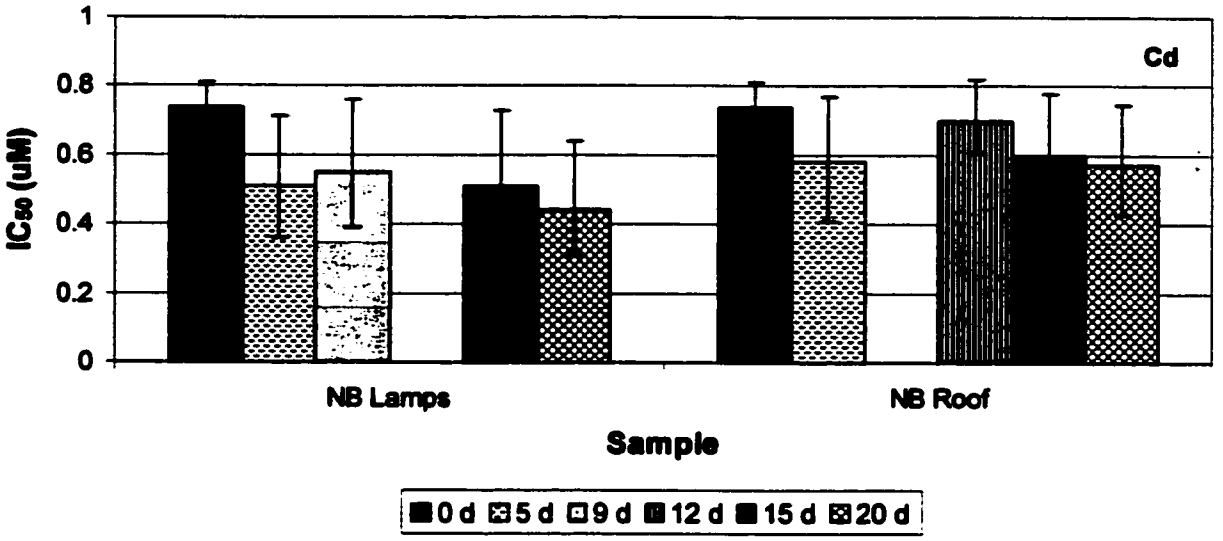
Table 3.1 Maximum concentrations of metals used to spike algal bioassays with Newington Bog water exposed to 0 d, 5 d, 9 d, 15 d and 20 d UVB radiation and 5 d, 12 d, 15 d and 20 d natural sunlight.

Metal	Maximum Concentration
Cu	200 µg/L (3.1 µM/L)
Ni	1000 µg/L (17.03 µM/L)
Cd	400 µg/L (3.6 µM/L)
Pb	600 ug/L (2.89 µM/L)

Figure 3.1. IC_{50} values (μM) and 95% confidence intervals as determined from bioassays using Newington Bog water exposed to UVB radiation then spiked with Cu, Ni, Cd and Pb.


Cu
IC50 (uM)
6
5
4
3
2
1
0
NB Lamps
NB Roof
Sample
0 d
5 d
9 d
12 d
15 d
20 d


Ni
IC50 (uM)
12
10
8
6
4
2
0
NB Lamps
NB Roof
Sample
0 d
5 d
9 d
12 d
15 d
20 d


Cd
IC50 (uM)
1
0.8
0.6
0.4
0.2
0
NB Lamps
NB Roof
Sample
0 d
5 d
9 d
12 d
15 d
20 d

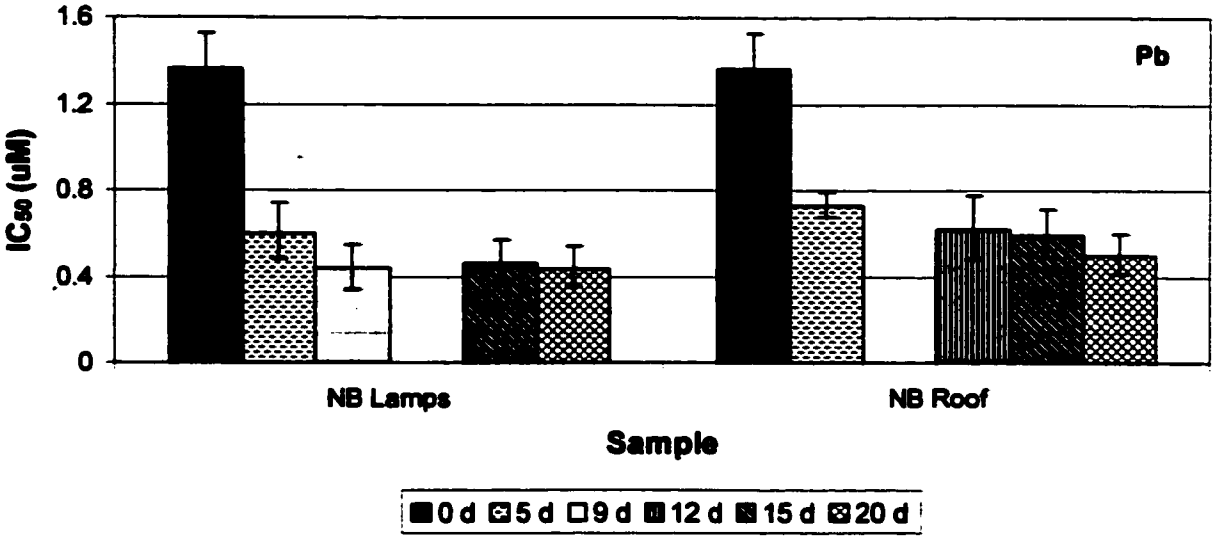

Pb
IC50 (uM)
1.6
1.2
0.8
0.4
0
NB Lamps
NB Roof
Sample
0 d
5 d
9 d
12 d
15 d
20 d

Figure 3.2 Log_{10}DOC concentration (mg C L^{-1}) vs. exposure time (days) in all Newington Bog subsamples after UVB (filled circles) and solar (open circles) irradiation.

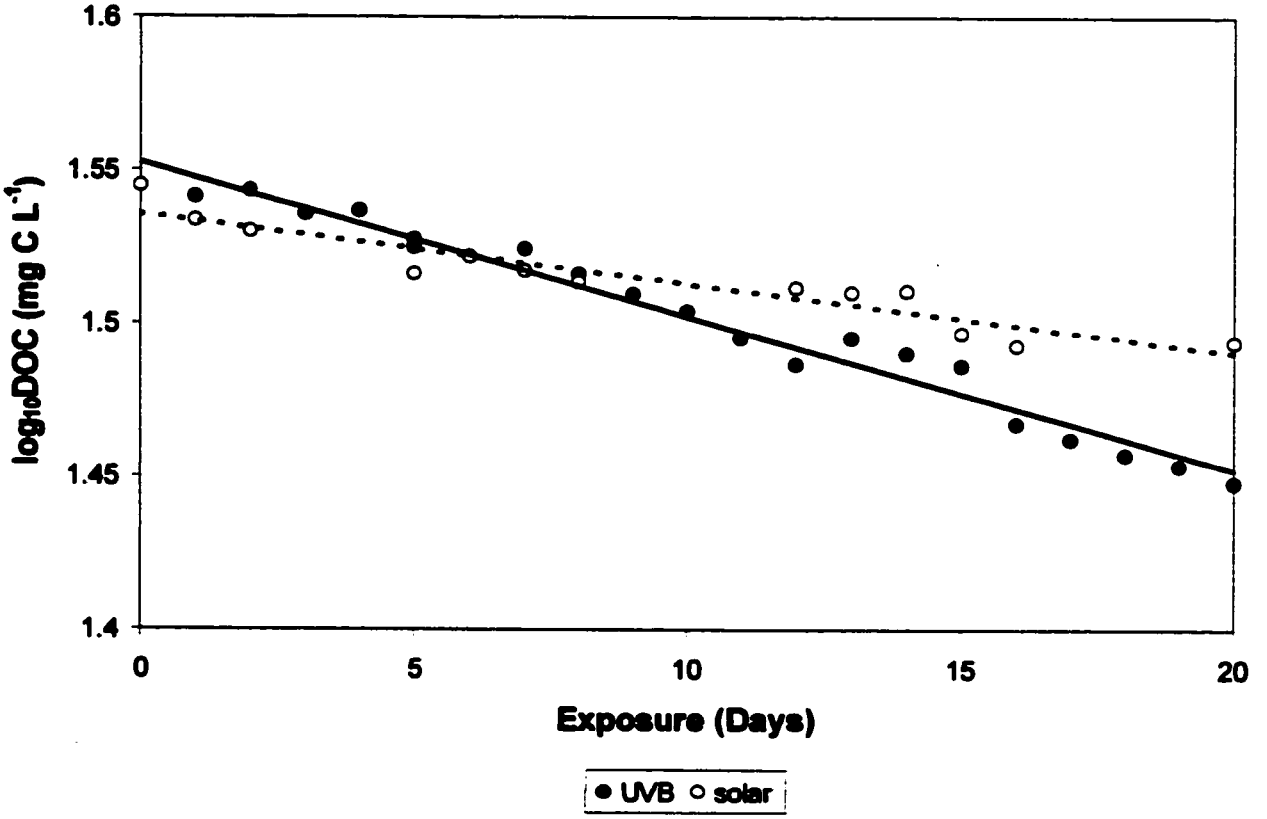

1.6
1.55
1.5
1.45
1.4
log10DOC (mg C L-1)
0
5
10
15
20
Exposure (Days)
UVB
solar

Figure 3.3 Log_{10}DOCFL (DOC fluorescence, qsu) vs. exposure time (days) in all Newington Bog subsamples after UVB (open circles) and solar (stars) irradiation.

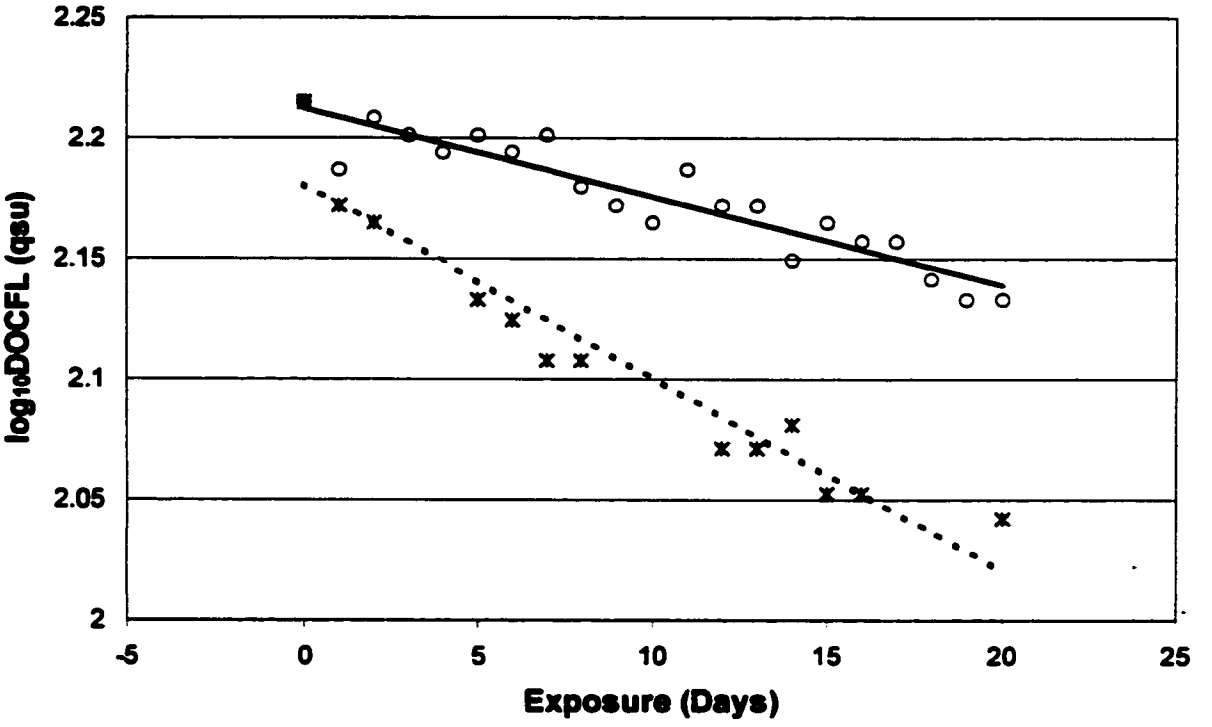
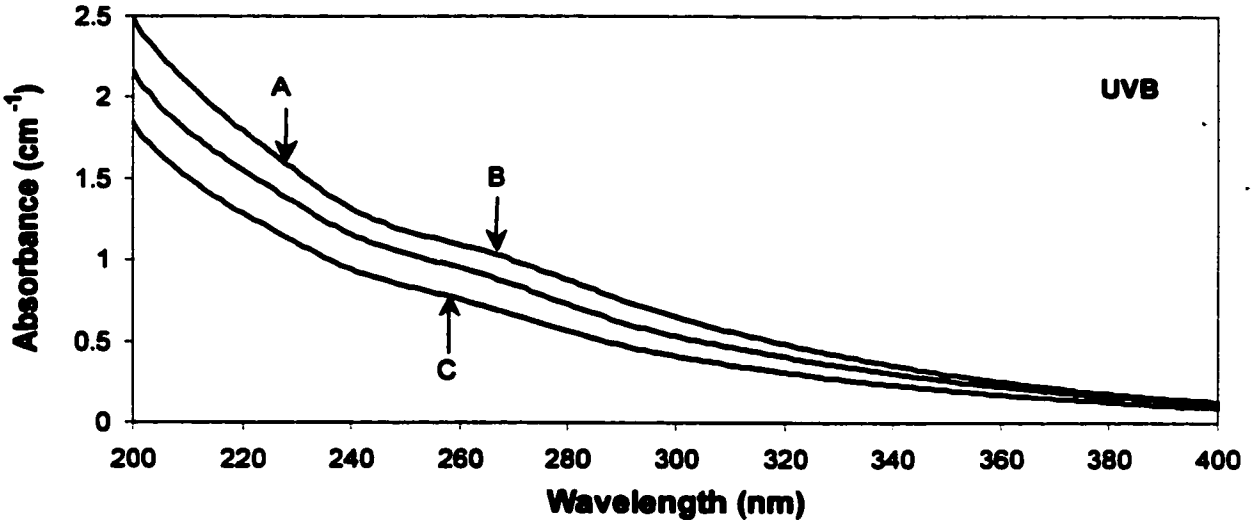

2.25
2.2
2.15
2.1
2.05
2
log10DOCFL (qsu)
-5
0
5
10
15
20
25
Exposure (Days)

Figure 3.4 Absorbance/mg C L^{-1} (cm^{-1}) vs. wavelength (nm) for 0 d, 9 d, 20 d UVB exposure and 0 d, 12 d, 20 d solar irradiation.


UVB
A
B
C
Absorbance (cm-1)
Wavelength (nm)
2.5
2
1.5
1
0.5
0
200
220
240
260
280
300
320
340
360
380
400

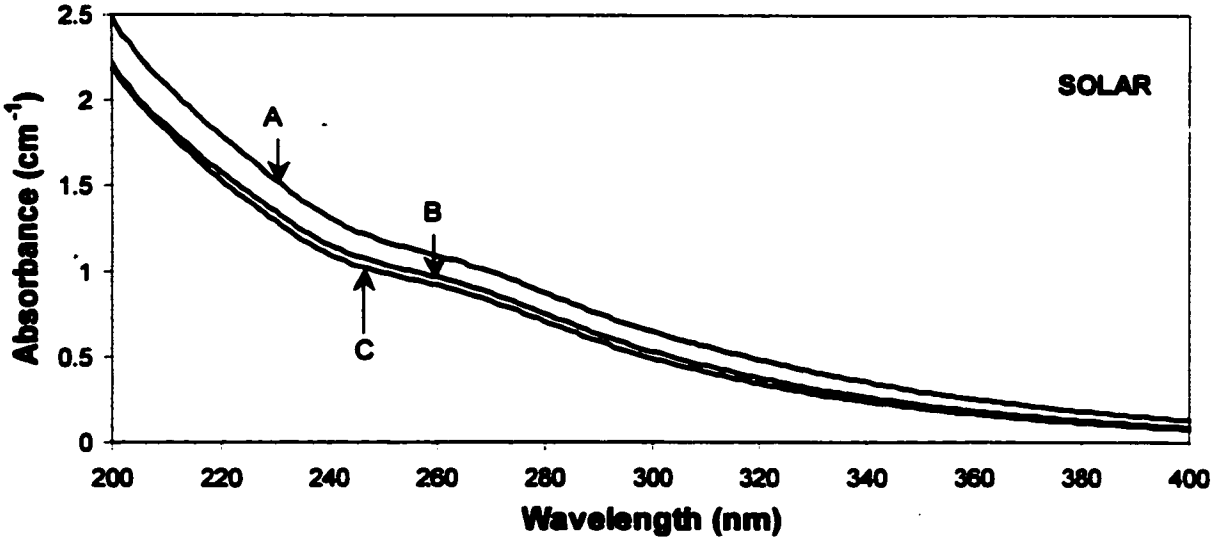

SOLAR
A
B
C
Absorbance (cm-1)
Wavelength (nm)
2.5
2
1.5
1
0.5
0
200
220
240
260
280
300
320
340
360
380
400

Figure 3.5 Absorbance (cm^{-1}) vs. wavelength (nm) for 5 mg NO_3^- L^{-1} in deionized water (A) and filtered (0.45 μm) samples from Lake Simcoe (B), St. Lawrence River (C), Grand River (D) and Newington Bog (E).

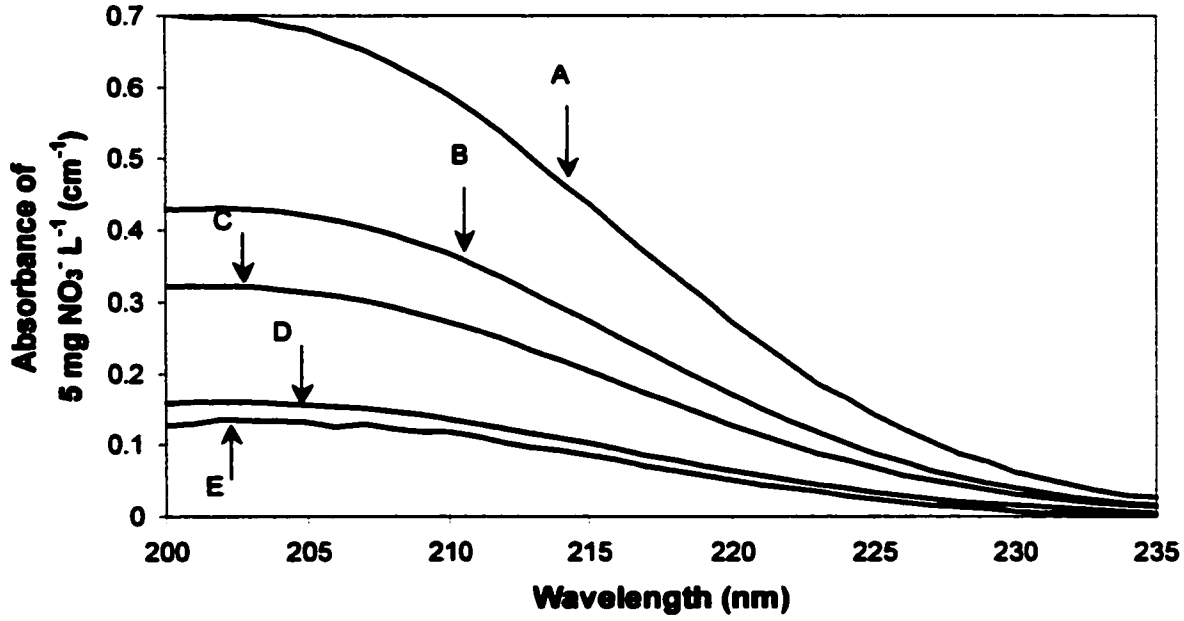
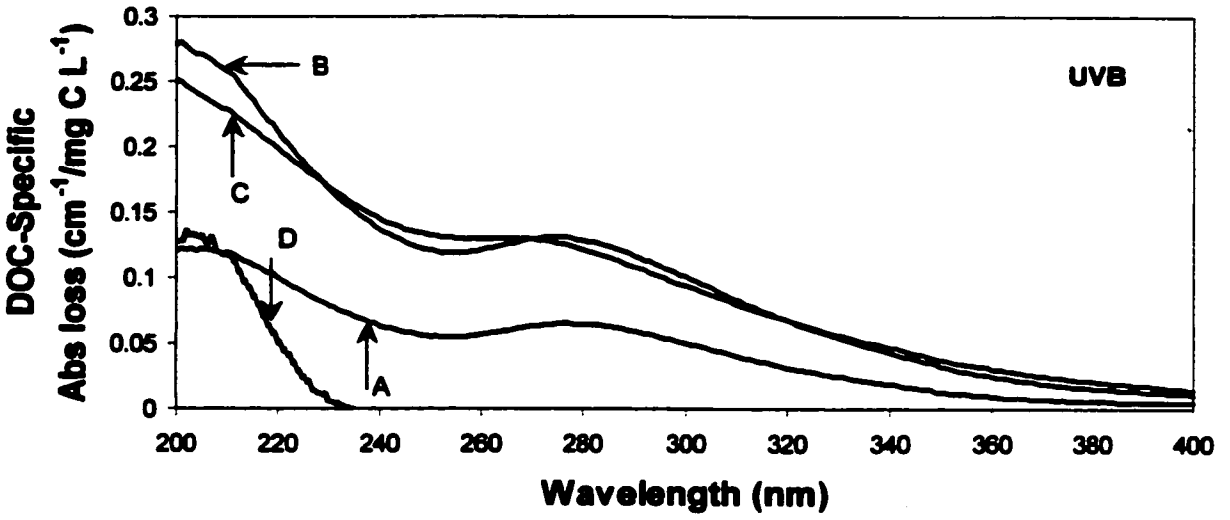

0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Absorbance of
5 mg NO3- L-1 (cm-1)
A
B
C
D
E
200
205
210
215
220
225
230
235
Wavelength (nm)

Figure 3.6 Absorbance loss/mg C L^{-1} (cm^{-1}) between 0 & 5 d (A), 0 & 9 d (B), and 0 & 20 d (C) for Newington Bog water exposed to UVB radiation in the laboratory, and between 0 & 5 d (E), 0 & 12 d (F) and 0 & 20 d (G) for Newington Bog water exposed to solar radiation. (D) is absorbance loss (cm^{-1}) vs. wavelength for 5 mg NO_3^- L^{-1} in unirradiated Newington Bog water.


DOC-Specific
Abs loss (cm-1/mg C L-1)
0.3
0.25
0.2
0.15
0.1
0.05
0
B
C
D
A
UVB
200
220
240
260
280
300
320
340
360
380
400
Wavelength (nm)

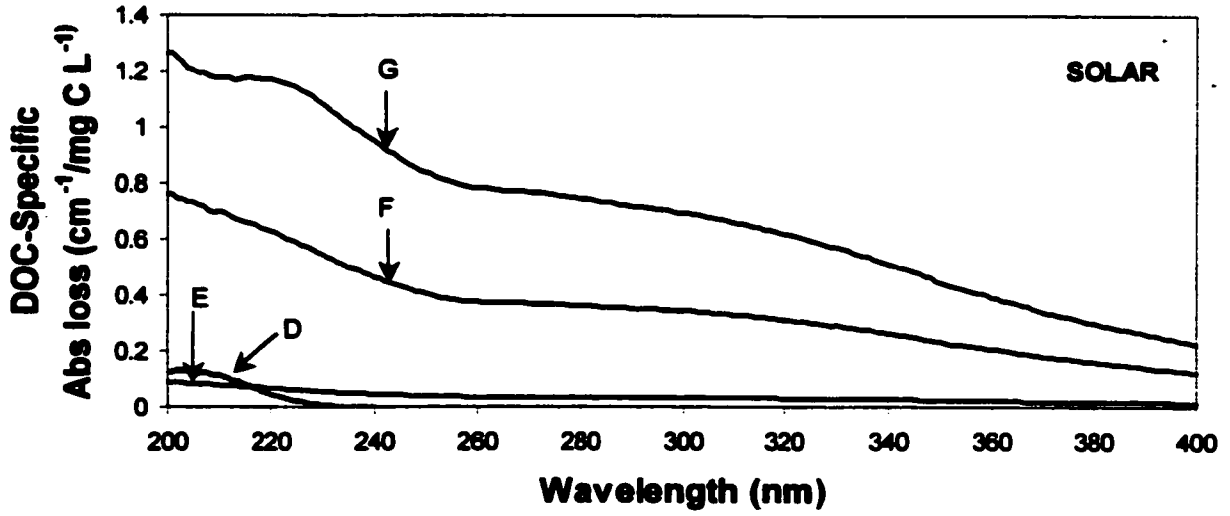
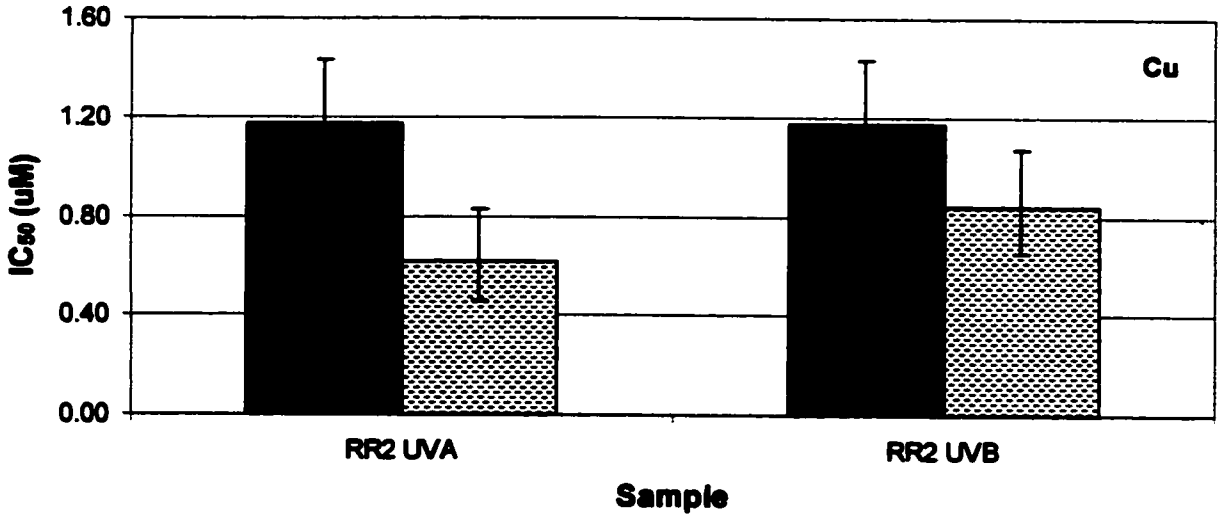
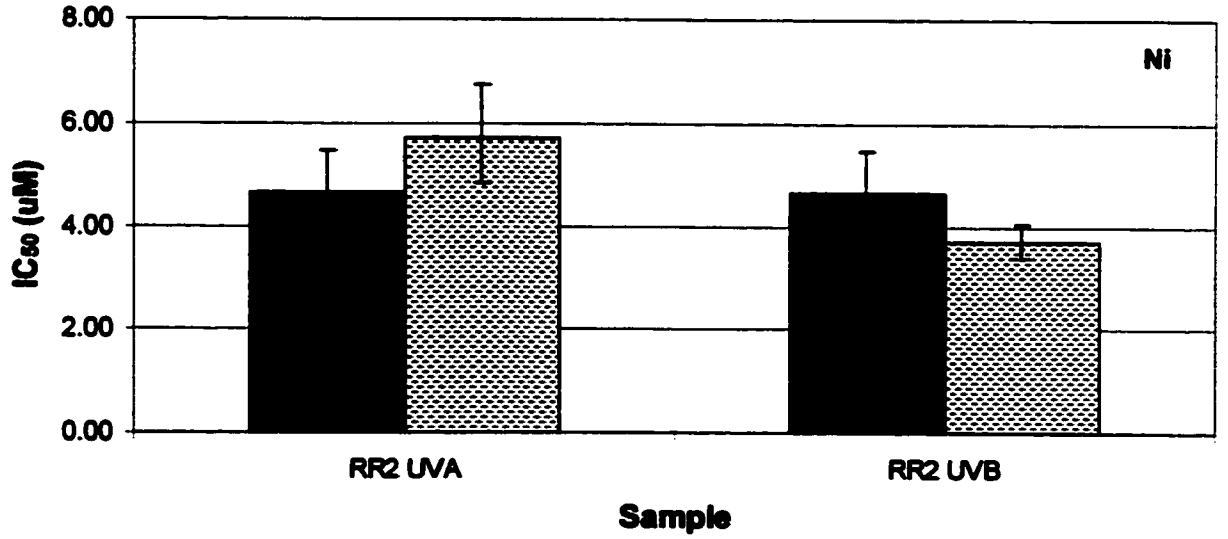
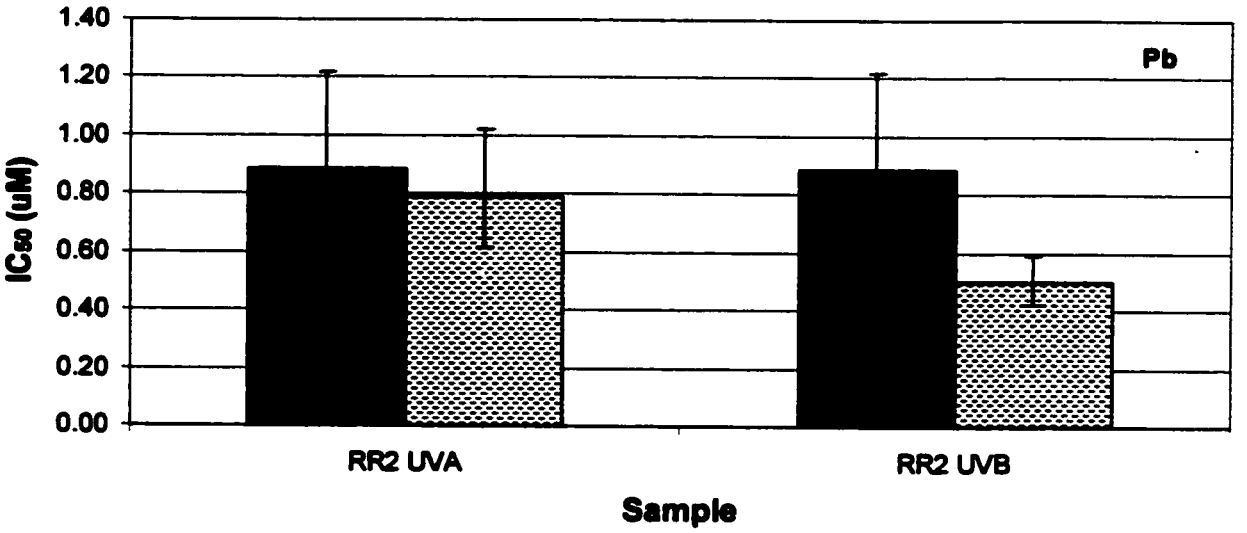

DOC-Specific
Abs loss (cm-1/mg C L-1)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
G
F
E
D
SOLAR
200
220
240
260
280
300
320
340
360
380
400
Wavelength (nm)

Figure 3.7 IC_{50} values (μM) and 95% confidence intervals as determined from bioassays using Raisin River (RR2) water exposed to UVB and UVA radiation for 0 d (gray) and 20 d (horizontal dashes), spiked with Cu, Ni, Pb and Cd.


1.60
1.20
0.80
0.40
0.00
IC50 (uM)
Cu
RR2 UVA
RR2 UVB
Sample


8.00
6.00
4.00
2.00
0.00
IC50 (uM)
Ni
RR2 UVA
RR2 UVB
Sample


1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
IC50 (uM)
Pb
RR2 UVA
RR2 UVB
Sample

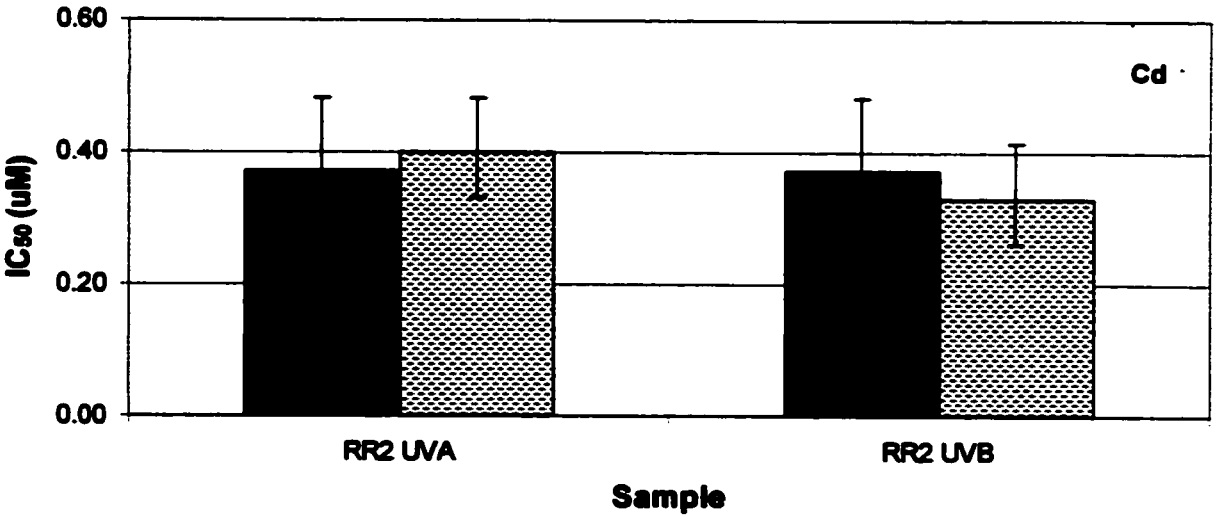

0.60
0.40
0.20
0.00
IC50 (uM)
Cd
RR2 UVA
RR2 UVB
Sample

Figure 3.8 Log_{10}DOC (mg C L^{-1}) vs. exposure time (days) in all Raisin River (RR2) samples after 20 d UVB irradiation (open circles) and 20 d UVA irradiation (stars).

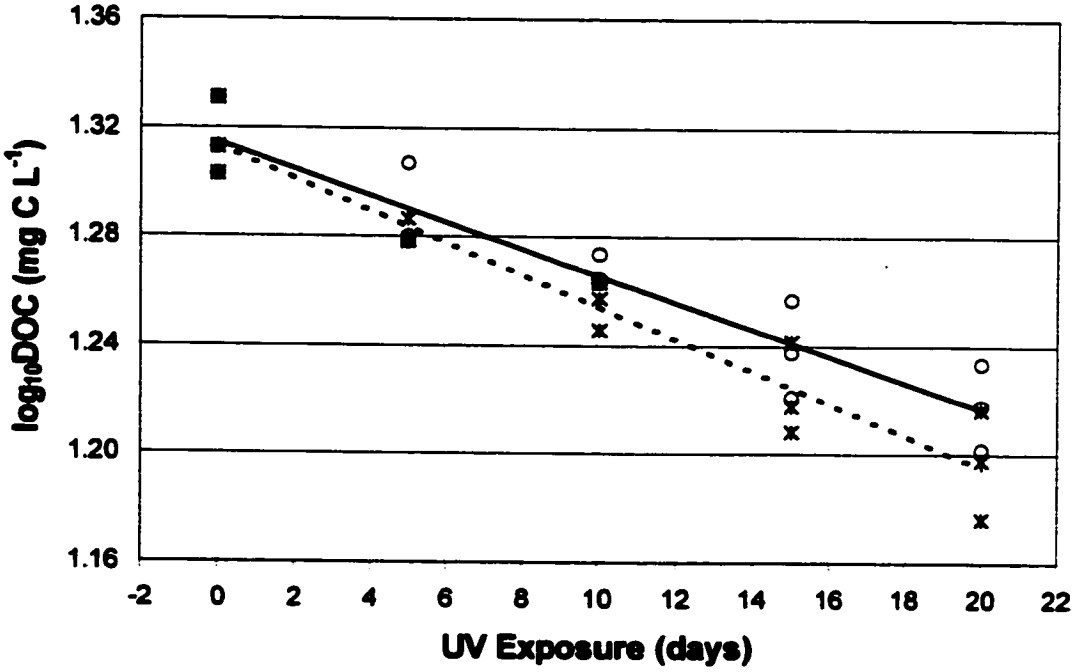

1.36
1.32
1.28
1.24
1.20
1.16
log10DOC (mg C L-1)
-2
0
2
4
6
8
10
12
14
16
18
20
22
UV Exposure (days)

Figure 3.9 DOC fluorescence (qsu) vs. exposure time (days) in Raisin River (RR2) samples after UVB irradiation (filled circles) and solar radiation (open circles).

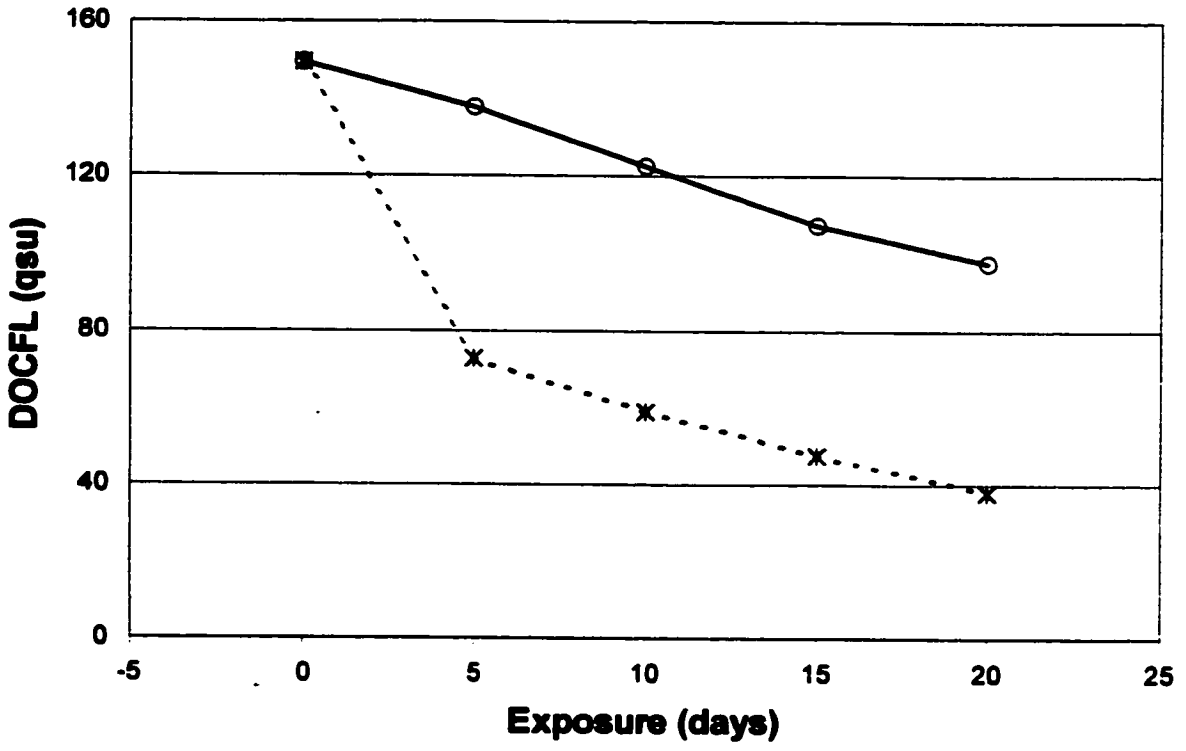
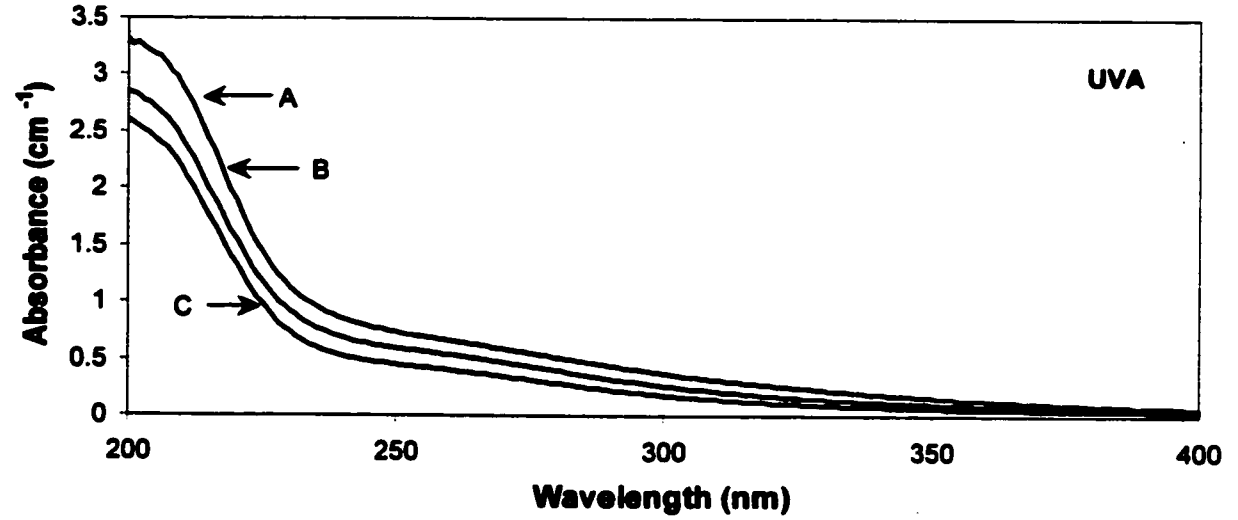

160
120
80
40
0
DOCFL (qsu)
-5
0
5
10
15
20
25
Exposure (days)

Figure 3.10 Absorbance (cm^{-1}) vs wavelength (nm) for Raisin River (RR2) water exposed to UVA and UVB radiation in the laboratory for 0 d (A), 10 d (B) and 20 d (C).


UVA
A
B
C
Absorbance (cm -1)
Wavelength (nm)
3.5
3
2.5
2
1.5
1
0.5
0
200
250
300
350
400

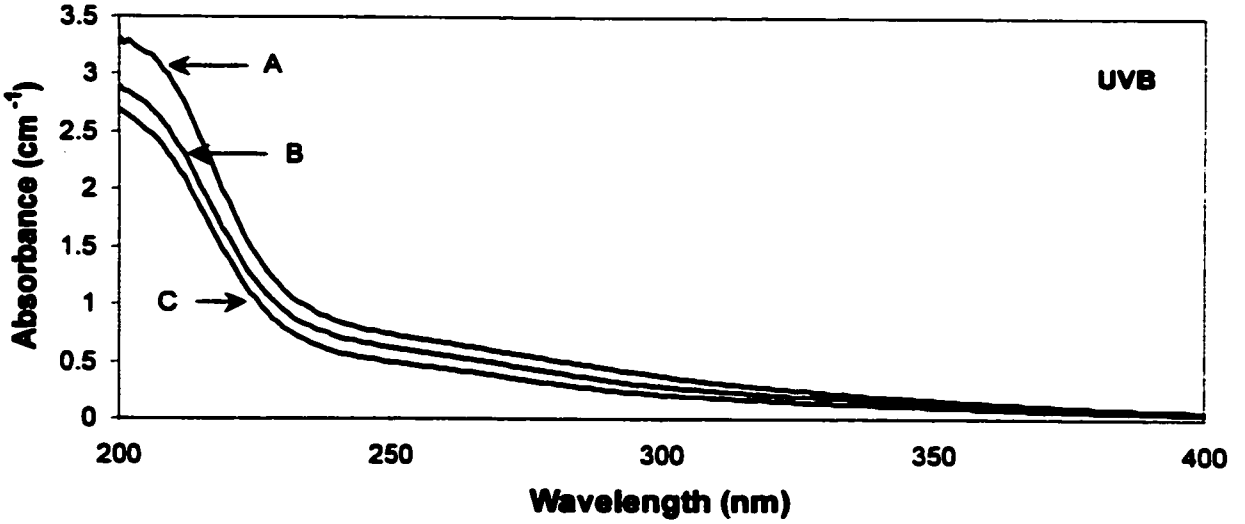
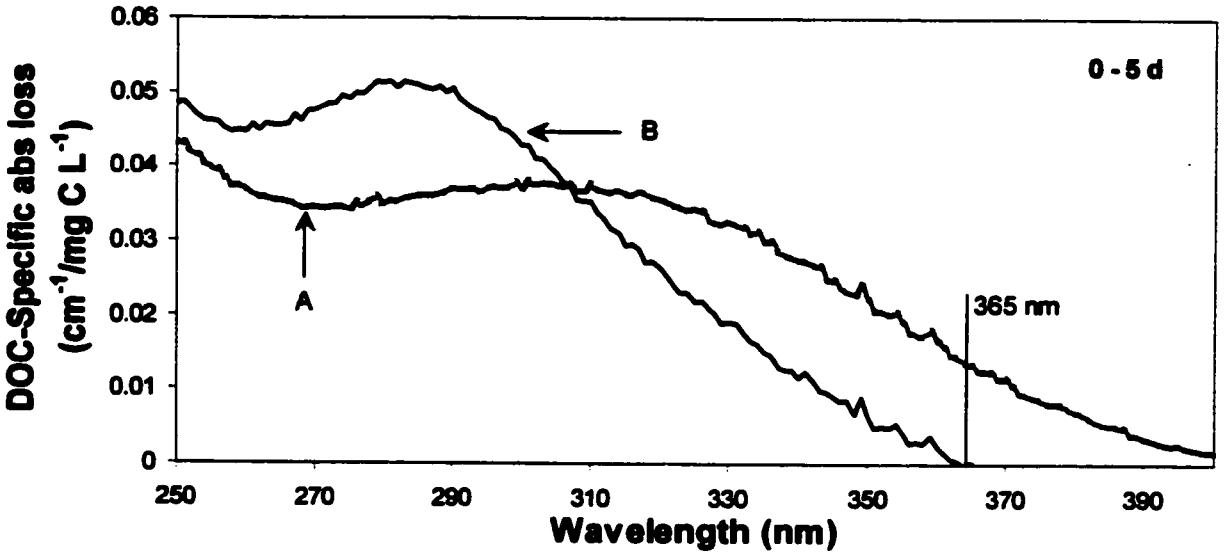
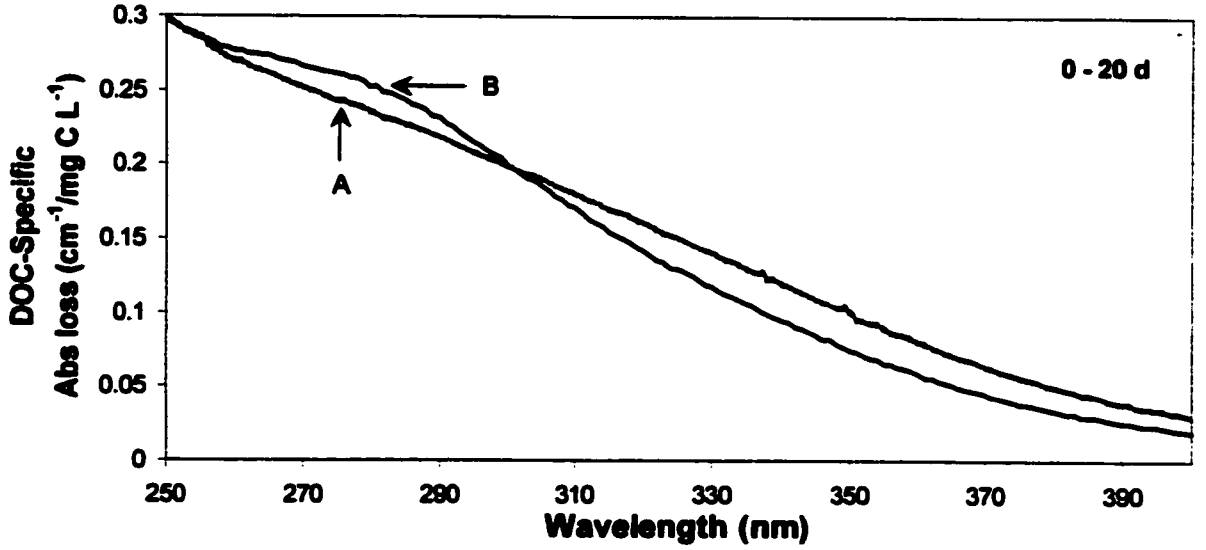

UVB
A
B
C
Absorbance (cm -1)
Wavelength (nm)
3.5
3
2.5
2
1.5
1
0.5
0
200
250
300
350
400

Figure 3.11 Absorbance loss /mg C L^{-1} (cm^{-1}) between 0 & 5 d and 0 & 20 d for Raisin River (RR2) water exposed to UVA (A) and UVB (B) radiation in the laboratory.


0 - 5 d
B
A
365 nm
DOC-Specific abs loss
(cm-1/mg C L-1)
0.06
0.05
0.04
0.03
0.02
0.01
0
250
270
290
310
330
350
370
390
Wavelength (nm)


0 - 20 d
B
A
DOC-Specific
Abs loss (cm-1/mg C L-1)
0.3
0.25
0.2
0.15
0.1
0.05
0
250
270
290
310
330
350
370
390
Wavelength (nm)

4.0 GENERAL DISCUSSION: IMPLICATIONS FOR ENVIRONMENTAL MANAGEMENT

The results of the research project will be important to decision-making regarding the generation and disposal of metal-containing waste products. They will also contribute to the growing body of knowledge surrounding protection of wetlands.

The Canadian Council of Ministers of the Environment (2001) has set out in its Water Quality Guidelines maximum concentrations of a wide range of toxic substances in natural fresh waters. Included in these guidelines are recommended maximum levels of Cd (0.017 $\mu g\ L^{-1}$), Cu (2-4 $\mu g\ L^{-1}$), Pb (1-7 $\mu g\ L^{-1}$), Ni (25-150 $\mu g\ L^{-1}$), and Zn (30 $\mu g\ L^{-1}$). (Maximum concentrations of Cd, Cu, Pb, Ni depend on water hardness.) The concentrations listed in the guidelines for Cd, Cu, Pb and Ni are well below the IC_{50} values determined in this study. In most cases (all values for St. Lawrence River, Lake Simcoe and Grand River, and some values for Raisin River after irradiation), IC_{50} for Zn was less than 20 $\mu g\ L^{-1}$, therefore the guideline may be excessively high for Zn.

Environmental Effects Monitoring (EEM) has been required of the pulp and paper industry since 1992. EEM was developed in response to the Pulp and Paper Effluent Regulations (PPER) and Metal Mining Effluent Regulations (MMER), under the *Fisheries Act*, and requires each regulated facility to design and implement its own monitoring plan, and report results and data to Environment Canada (Environment Canada, 2002).

A similar initiative has very recently been implemented in the mining industry. The Metal Mining Liquid Effluent Regulations (MMLER) were promulgated under the Fisheries Act in 1977 for the purpose of assessing and controling effects of mining effluents on fish, fish habitat and the human use of fisheries resources. MMLER set out effluent limits for As, Cu, Pb, Ni, Zn, total suspended matter, radium 226 and pH, but did not apply to metal mines and mills that opened, reopened or expanded prior to 1977. The Assessment of the Aquatic Effects of Mining in Canada (AQUAMIN) began in 1993, and constituted the basis for an EEM program for the mining sector in Canada. The AQUAMIN final report, released in October, 1996, found that effects from mining activity ranged from no detectable effects to effects tens of kilometers downstream, and contaminants of concern included Zn, Cd, Pb, Cu, among others. The causes of these effects were not always readily identifiable (Environment Canada, 2002).

AQUAMIN further determined that there was no nationally consistent monitoring framework in Canada, and recommended that MMLER be revised, that EEM be established for the mining sector, and that site-specific requirements be put in place to ensure that local receiving environments are adequately protected.

The new Metal Mining Effluent Regulations (MMER) were registered on June 6, 2002, and include a requirement for metal mines in Canada to conduct EEM. Although the requirements for EEM have not yet been published, it is proposed that they be site-specific and include biological monitoring (of fish and benthic invertebrates), and effluent and

water monitoring (including effluent characterization, water quality monitoring and sublethal toxicity testing) (Environment Canada, 2002).

The research reported in this study is most relevant to the site-specificity of EEM, and to the location of sampling sites. If site-specific studies take into account DOC concentrations in receiving waters, then the results of biological monitoring, water quality monitoring and toxicity testing could be misleading. In situations where receiving waters contain high levels of fresh DOC from wetlands, there could be a tendency to allow higher concentrations of metals in effluents, as a higher proportion of total metals would be chelated by DOC. As the water moves downstream, however, DOC is exposed to UV radiation causing metals to be released and become bioavailable. Site-specific requirements should, therefore, be developed bearing in mind the downstream alteration and degradation of DOC by UV, and decisions regarding sample sites for toxicity tests, biological monitoring and water quality monitoring should take into account the potential for changes in metal speciation over long distances downstream.

At present, no national policies exist in Canada to monitor or regulate effluents from industries other than mining and pulp and paper, although metal-containing effluents are generated from operations ranging from food preparation to chemical manufacturing, production and use of pesticides, coal burning, electroplating, etc. (Canadian Council of Ministers of the Environment, 2001). Some industries are self-regulating, and provincial or municipal bylaws vary among jurisdictions.

Destruction of wetlands greatly reduces concentrations of DOC in rivers, streams and lakes. The results of this study should provide support to efforts to protect wetlands from direct and indirect anthropogenic disruption. At present, Environment Canada's Federal Water Policy (Environment Canada, 1987) stipulates that the federal government will conserve and manage wetlands of importance on federally owned or regulated lands, and co-operate with other governments in conserving and managing wetlands which serve important hydrological roles associated with improving water quality, along with a number of other measures aimed at minimizing negative impacts on wetlands and increasing public awareness of the importance of wetland conservation. This study provides support for initiatives of government at all levels to conserve wetlands and promote awareness of their importance.

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APPENDIX A

IC_{50} values and 95% confidence limits for algal bioassays.

Sample location	Metal	UVB exposure (days)	IC_{50} µM	Upper 95% confidence limit	Lower 95% confidence limit	R^2
RR1	Pb	0	1.74	2.15	1.41	0.89
		5	0.98	1.46	0.66	0.88
		10	0.76	0.89	0.65	0.94
	Cu	0	2.88	3.52	2.36	0.75
		5	2.01	2.30	1.75	0.92
		10	2.03	2.22	1.85	0.97
	Zn	0	0.67	1.02	0.44	0.91
		5	0.43	0.95	0.19	0.85
		10	0.15	0.19	0.11	0.95
	Ni	0	6.98	8.43	5.79	0.86
		5	7.71	10.06	5.91	0.77
		10	7.04	8.00	6.21	0.93
	Cd	0	0.46	0.58	0.39	0.89
		5	0.42	0.86	0.21	0.74
		10	0.47	0.55	0.40	0.95
	Co	0	7.68	11.64	5.06	0.89
		5	4.21	6.67	2.67	0.85
		10	3.54	5.26	3.03	0.75
L. Simcoe	Pb	0	0.25	0.62	0.15	0.67
		5	0.11	0.21	0.06	0.79
		10	0.06	0.12	0.03	0.71
	Cu	0	0.24	0.32	0.17	0.86
		5	0.24	0.30	0.19	0.86
		10	0.21	0.33	0.13	0.87
	Zn	0	0.17	0.37	0.08	0.75
		5	0.14	0.21	0.09	0.89
		10	0.23	0.58	0.09	0.35
	Ni	0	2.01	2.61	1.56	0.90
		5	1.95	2.69	1.41	0.85
		10	1.88	3.03	1.17	0.49
	Cd	0	0.14	0.41	0.05	0.54
		5	0.17	0.27	0.11	0.87
		10	0.45	0.51	0.39	0.75
	Co	0	1.26	4.82	0.33	0.32
		5	1.04	1.76	0.62	0.75
		10	0.23	0.79	0.07	0.44

Grand R.	Pb	0	0.44	0.52	0.37	0.98
		5	0.37	0.69	0.20	0.76
		10	0.54	0.76	0.38	0.92
	Cu	0	0.99	1.11	0.88	0.92
		5	0.59	0.68	0.51	0.92
		10	0.74	0.81	0.68	0.96
	Zn	0	0.24	0.32	0.17	0.94
		5	0.2	0.32	0.13	0.87
		10	0.15	0.25	0.09	0.84
	Ni	0	7.31	9.51	5.61	0.76
		5	6.93	8.96	5.36	0.87
		10	4.72	5.75	3.88	0.94
	Cd	0	0.32	0.38	0.27	0.96
		5	0.31	0.35	0.27	0.98
		10	0.36	0.47	0.28	0.91
	Co	0	4.4	5.58	3.47	0.91
		5	3.05	3.94	2.37	0.87
		10	4.98	7.96	3.12	0.70
SLR	Pb	0	0.08	0.11	0.06	0.93
		5	0.2	0.29	0.14	0.91
		10	0.27	0.47	0.15	0.66
	Cu	0	0.34	0.65	0.18	0.45
		5	0.23	0.3	0.17	0.86
		10	0.08	0.1	0.06	0.91
	Zn	0	0.17	0.23	0.12	0.94
		5	0.2	0.32	0.12	0.92
		10	0.31	0.39	0.25	0.86
	Ni	0	2.07	3.15	1.36	0.77
		5	3.22	4.21	2.46	0.56
		10	0.91	1.23	0.67	0.87
	Cd	0	0.08	0.11	0.06	0.93
		5	0.24	0.33	0.18	0.83
		10	0.25	0.4	0.16	0.43
	Co	0	0.38	0.5	0.29	0.95
		5	1.04	1.41	0.76	0.83
		10	3.48	7.85	1.54	0.39
NB Lamps	Pb	0	1.19	1.36	1.04	0.95
		5	0.6	0.74	0.48	0.97
		9	0.44	0.55	0.34	0.97
		15	0.46	0.57	0.37	0.96
		20	0.43	0.54	0.35	0.96
NB Roof	Pb	5	0.73	0.79	0.68	0.99
		12	0.62	0.78	0.48	0.95
		15	0.59	0.71	0.49	0.97
		20	0.5	0.6	0.41	0.97
NB Lamps	Cu	0	4.65	5.84	3.7	0.83
		5	2.9	3.31	2.55	0.84
		9	2.41	2.65	2.19	0.95
		15	2.07	2.29	1.88	0.94
		20	1.73	1.97	1.52	0.93

NB Roof	Cu	5	2.52	2.97	2.14	0.88
		12	2.38	2.63	2.16	0.92
		15	1.96	2.25	1.7	0.89
		20	2.36	2.6	2.15	0.93
NB Lamps	Ni	0	9.7	10.98	8.57	0.94
		5	7.99	8.79	7.26	0.96
		9	7.76	8.72	6.91	0.97
		15	7.74	8.67	6.91	0.97
		20	6.28	7.2	5.49	0.93
NB Roof	Ni	5	8.31	8.89	7.78	0.97
		12	8.35	9.3	7.49	0.93
		15	8.34	8.85	7.85	0.98
		20	7.91	9.03	6.93	0.95
NB Lamps	Cd	0	0.74	0.81	0.68	0.99
		5	0.51	0.71	0.36	0.91
		9	0.55	0.76	0.39	0.92
		15	0.51	0.73	0.36	0.9
		20	0.44	0.64	0.31	0.9
NB Roof	Cd	5	0.58	0.77	0.41	0.93
		12	0.7	0.82	0.61	0.98
		15	0.6	0.78	0.47	0.95
		20	0.57	0.75	0.43	0.94
RR2	Cu	0	1.17	1.43	0.96	0.97
		20 UVA	0.62	0.83	0.46	0.93
		20 UVB	0.84	1.07	0.65	0.95
	Ni	0	4.63	5.44	3.95	0.86
		20 UVA	5.71	6.76	4.82	0.89
		20 UVB	3.70	4.04	3.40	0.96
	Cd	0	0.37	0.48	0.29	0.78
		20 UVA	0.40	0.48	0.33	0.91
		20 UVB	0.33	0.41	0.26	0.92
	Pb	0	0.88	1.22	0.64	0.87
		20 UVA	0.79	1.02	0.62	0.78
		20 UVB	0.50	0.60	0.42	0.95

APPENDIX B

Anions

				Concentration [mg/L]		
Name	Fluoride	Chloride	Nitrite	Sulphate	Nitrate	Phosphate
Raisin River 0 d	0.0845	31.362	0.0299	5.839	0.933	n.a.
Grand River 0 d	0.096	21.390	0.026	7.450	2.490	n.a.
Lake Simcoe 0 d	0.069	3.451	n.a.	2.075	n.a.	n.a.
St. Lawrence 0 d	0.1188	21.4415	n.a.	25.7292	1.1448	n.a.
Newington Bog 0 d	0.1200	1.6939	0.0375	1.6240	5.3536	n.a.
Newington Bog 9 d UVB	0.1773	1.7425	5.5145	1.8890	0.2469	n.a.
Newington Bog 20 d UVB	0.2129	1.7002	5.5093	2.2130	0.4725	n.a.
Newington Bog 12 d sun	0.1701	1.8033	0.0534	1.9433	0.4749	n.a.
Newington Bog 20 d sun	0.1879	1.7280	0.2100	1.9596	0.8673	n.a.

Cations (Ion Chromatography)

				Concentration [mg/L]				
Name	Lithium	Sodium	Ammonium	Potassium	Magnesium	Manganese	Calcium	Strontium
Raisin River 0 d	0.0019	16.207	----	0.829	5.452	0.0764	70.583	0.277
Grand River 0 d	0.0020	10.890	----	0.8593	19.510	n.a.	60.860	0.120
Lake Simcoe 0 d	0.0020	16.500	----	1.870	7.170	n.a.	41.800	0.150
St. Lawrence 0 d	0.0031	12.1025	n.a.	1.4411	7.5553	n.a.	28.8635	n.a.
Newington Bog 0 d	0.0048	5.7695	n.a.	1.7081	8.4053	n.a.	25.4006	0.3886
Newington Bog 9 d UVB	0.0048	5.8856	n.a.	1.8367	8.5050	n.a.	25.6899	0.3104
Newington Bog 20 d UVB	0.0048	5.8422	n.a.	1.7734	8.5287	n.a.	25.7225	0.5144
Newington Bog 12 d sun	0.0046	5.7996	1.5187	1.7848	8.4354	n.a.	25.6138	0.4509
Newington Bog 20 d sun	0.0046	5.7880	1.4637	1.7605	8.3717	n.a.	25.4726	0.4996

Cations (ICPOES and ICPMS)

				Concentration [mg/L]		
Name	Iron	Cobalt	Copper	Nickel	Lead	Zinc
Raisin River 0 d	0.350	0.00030	0.0017	0.0022	0.0023	0.006
Grand River 0 d	0.070	0.00026	0.0016	0.0020	0.0012	0.003
Lake Simcoe 0 d	0.940	0.00012	0.0008	0.0012	n.a.	n.a.