# TECHNICAL EVALUATION OF MONITORING METHODS USING MACROPHYTES, PHYTOPLANKTON AND PERIPHYTON TO ASSESS THE IMPACTS OF MINE EFFLUENTS ON THE AQUATIC ENVIRONMENT

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## EXECUTIVE SUMMARY

The Aquatic Effects Technology Evaluation Program, AETE, has been established to assist the Canadian mining industry in meeting its environmental effects monitoring and related requirements, in a cost-effective a manner as possible. The program is coordinated by the Canadian Center for Mineral and Energy Technology (CANMET). The present report is a technical evaluation of monitoring methods using macrophytes, phytoplankton and periphyton to assess the impacts of mine effluents on the aquatic environment.

A wide literature review was undertaken, and for each of macrophytes, phytoplankton and periphyton, case studies reporting their use as biomonitors of contaminated environments, including areas near mining activities, are described. Then, a critical evaluation of the biomonitoring methods used was done. Other biomonitoring methods, having high potential but not yet investigated in the field, are also identified.

Macrophytes appear to have an interesting potential as biomonitoring organisms to assess the impacts of mine effluents on the aquatic environment. Their usefulness until now has been under-estimated. Many macrophyte species conform very well to many of the criteria listed for an «ideal» biomonitor organism: they are sedentary, visible to the naked eye, easy to collect and to handle, easy to identify in the field, they concentrate metals in their tissues and reflect the environmental contamination. Metals taken up by rooted submerged macrophyte species represent the bioavailable, free-metal ion concentrations in the sediment interstitial water, as well as metal contamination in the water column, if present. Plant parts to be analysed, cleaning methods and methods to deal with the variability of the results are discussed. Although biochemical indicators, such as phytochelatins and enzyme induction (peroxidase activity), have not yet been used in the field with macrophytes to assess metal contamination, their possible use as biomonitoring tools is briefly discussed. Further work needs to be done, outside of AETE, before the routine use of macrophytes as biomonitor organisms can be effectively applied in the field. It would be interesting to collect and analyse for metals submerged rooted species along with other more routinely sampled benthic organisms used to monitor the environment, such as aquatic insects and mollusks, and to determine for all sampling sites the free-metal ion concentration at the sediment/water interface, which is the best current indicator of bioavailable metal for these organisms; and then to compare the usefulness of using macrophytes with the other more «standard» biomonitor organisms. *Eriocaulon* *septangulare*, *Eleocharis acicularis* and *Potamogeton richardsonii* appear to be particularly promising candidates for biomonitoring in the Canadian mining environment.

Because phytoplankton are very responsive to changes in water quality and are at the base of lake trophic chain, phytoplankton could be an effective and meaningful biomonitoring tool. Only methods based on biomass changes and species shifts have so far been applied to monitoring of mine sites. This review has identified several approaches that promise to be more powerful than these traditional ones: 1) community canonical analysis, 2) size distribution, 3) pigment analysis, 4) phytochelatin analysis, 5) diatom deformities, 6) tests based on community induced tolerance. All these approaches need field testing to verify their applicability and generality. The potentials and the limitations of these techniques in view of their integration into a cost effective monitoring program are discussed.

In aquatic ecosystems, the importance of the periphytic communities is widely recognized. Periphyton is a functional system where autotrophic and heterotrophic processes take place. It plays a role at the interface between substrata and the surrounding water. This community may, consequently, influence biogeochemical pathways and the dynamics of the ecosystem. For studying environmental perturbations from mine effluents, periphyton represents many advantages. Since it is non mobile, it is easy to sample and integrate effects of environmental variables. In addition, periphyton has a rapid response to disturbance which may create modifications of community structure and functioning. Even under very harsh disturbances, periphyton doesn’t disappear completely. On the other hand, periphytic communities are strongly influenced by the variability of physical conditions in the field. In addition, the complexity and heterogeneity of this community, and the lack of methodological standardization, restrict its use in impact studies of mine effluents and explain why periphyton is less studied than phytoplankton. A literature review notes the interest for studies on changes in the kind of species or their relative abundance and distribution. Indeed, the structure of the periphytic community reflects the competitive capacity of the species forming it and can, consequently, serve as a source of integrated information which can serve as a sensitive indicator of metal or acid stress.

## SOMMAIRE

Le programme d'Évaluation des Techniques de Mesures d'Impacts en Milieu Aquatique (ÉTIMA) a été mis sur pied afin d'assister l'industrie minière canadienne dans la sélection de techniques efficaces et peu coûteuses pour la surveillance environnementale («biomonitoring») des effets des métaux toxiques en milieu naturel. Le programme est géré par le Centre canadien de la technologie des minéraux et de l'énergie (CANMET). Le présent rapport constitue une évaluation technique des méthodes de surveillance environnementale utilisant les macrophytes, le phytoplankton et le périphyton pour mesurer les impacts des effluents miniers sur l'environnement aquatique.

Une large revue de la littérature concernant les macrophytes, le phytoplancton ainsi que le périphyton comme organismes sentinelles d'environnements contaminés par les métaux, y compris ceux entourant les sites miniers, est rapportée. Une évaluation critique des méthodes de surveillance environnementale utilisées est ensuite réalisée, et d'autres méthodes potentielles, mais n'ayant encore fait l'objet d'aucune étude de terrain, sont identifiées.

Les macrophytes présentent un potentiel intéressant comme organismes sentinelles afin de mesurer les impacts des effluents miniers sur l'environnement aquatique. Leur utilisation a été sous-estimée jusqu'à présent. Plusieurs espèces de macrophytes se conforment à plusieurs des critères énumérés auquel doit répondre un organisme sentinelle «idéal»: elles sont sédentaires, visibles à l'oeil nu, faciles à échantillonner et à manipuler, elles concentrent les métaux dans leurs tissus et réflètent la contamination environnementale. Les concentrations en métaux retrouvés dans les tissus d'espèces de macrophytes enracinés submergés réflètent les concentrations biodisponibles d'ions libres dans les eaux interstitielles des sédiments, ainsi que la contamination possible de la colonne d'eau. Quelle partie de la plante utiliser, des méthodes afin d'enlever la contamination possible sur les racines et les parties vertes ainsi que pour réduire la variabilité des résultats sont discutés. Même si les indicateurs biochimiques de stress (comme les phytochélatines et l'activité enzymatique, principalement de la peroxidase) n'ont pas encore été utilisés chez les macrophytes comme moyen de surveillance environnementale sur le terrain, le potentiel

qu'ils représentent est brièvement rapporté. D'autres études sur le terrain, effectuées à l'extérieur du programme ÉTIMA, seraient nécessaires avant que l'usage routinier des macrophytes comme organismes sentinelles soit applicable en milieu minier. Il serait intéressant d'échantillonner et d'analyser pour les métaux des espèces de macrophytes enracinés submergés récoltées avec d'autres organismes sentinelles benthiques plus fréquemment utilisés comme organismes sentinelles d'environnements contaminés, tels que les insectes aquatiques et les mollusques, et d'estimer à tous les sites d'échantillonnage les concentrations d'ions libres des métaux à l'interface sédiment/eau, lesquelles représentent le meilleur indice de la partie biodisponible des métaux totaux pour ces organismes, et alors de comparer l'utilité des macrophytes avec celle des autres organismes sentinelles «standards». Pour le milieu minier canadien, *Eriocaulon septangulare*, *Eleocharis acicularis* et *Potamogeton richardsonii* semblent être des candidats prometteurs à titre d'organismes bioindicateurs.

Étant donné que le phytoplancton répond bien aux changements de la qualité des eaux et qu'il se situe à la base de la chaîne trophique, il peut servir comme un outil efficace et significatif pour le suivi biologique. Jusqu'ici, seulement des méthodes basées sur les changements de biomasse et d'espèces ont été utilisées pour la surveillance environnementale des sites miniers. Cette revue de littérature a permis d'identifier plusieurs approches qui pourraient être plus puissantes que les traditionelles: 1) analyse canonique de la communauté, 2) distribution en taille, 3) analyse des pigments, 4) dosage des phytochélatines, 5) difformité des diatomés, et 6) test basé sur la tolérance induite des communautés. Toutes ces approches doivent être vérifiées pour tester leur applicabilité et leur généralité. Le potentiel et les limites de ces techniques en vue de leur intégration dans un programme de surveillance environnementale sont discutés.

Dans les écosystèmes aquatiques, l'importance des communautés périphytiques est largement reconnue. Le périphyton constitue un système fonctionnel où se déroulent des processus autotrophes et hétérotrophes. Il joue le rôle d'interface entre le substrat et l'eau environnante. Cette communauté peut donc influer sur les voies biogéochimiques et sur la dynamique de l'ensemble de l'écosystème. Pour l'étude de perturbations environnementales résultant de la présence d'effluents miniers, le périphyton représente plusieurs avantages. Étant fixé, il est relativement facile à échantillonner et intègre les effets des variables de l'environnement. De plus, il montre un temps de réponse court aux perturbations qui peuvent provoquer des modifications de la structure et du fonctionnement de ces communautés. Par ailleurs, même lors de perturbations très sévères, le périphyton ne disparaît jamais complètement. Cependant, les communautés périphytiques sont fortement influencées par la variabilité des conditions physiques du milieu. De plus, la complexité et l'hétérogénéité de cette communauté, ainsi que l'absence d'une standardisation méthodologique, limitent encore son utilisation dans les études d'impact des effluents miniers et expliquent que le périphyton ait été moins étudié que le phytoplancton. La revue de littérature a cependant fait ressortir l'intérêt des études sur les changements d'espèces ou de leur abondance relative et de leur distribution. En effet, la structure de la communauté périphytique reflète la capacité compétitive des espèces qui la composent et peut donc fournir une source d'informations intégrées pouvant servir d'indicateur sensible à la présence des métaux.

# INTRODUCTION

The Aquatic Effects Technology Evaluation Program, AETE, has been established to assist the Canadian mining industry in meeting its environmental effects monitoring and related requirements, in a cost-effective a manner as possible. The program is coordinated by the Canadian Center for Mineral and Energy Technology (CANMET).

Biomonitoring methods using macrophytes, phytoplankton and periphyton have the potential to be useful tools for monitoring impacts of mine effluents on the aquatic environment. In order to evaluate the usefulness of these techniques for the Canadian mining industry, the AETE Technical Committee requested that a technical evaluation of monitoring methods using macrophytes, phytoplankton and periphyton be undertaken.

The overall objective of this technical evaluation is to review established and emerging monitoring methods using macrophytes, phytoplankton and periphyton, and to make recommendations as to the usefulness and cost-effectiveness of these methods. Each of the macrophytes, phytoplankton and periphyton was evaluated separately by different authors. A literature review of the existing monitoring methods in used in different environmental biomonitoring studies in Canada and elsewhere were done, with examples, highlighting their advantages, disadvantages and limitations, and other biomonitoring methods, having high potential but not yet investigated in the field, are identified. A last chapter, written by another author, critically evaluate the usefulness of these monitoring tools against a number of criteria, including ecological relevance, validation, practical limitations and commercial availability. Finally, a one-page summary of each of the monitoring methods reviewed is presented.

# 1. MACROPHYTES

by

Louise St-Cyr

## 1.1 OVERVIEW OF BIOMONITORING USING MACROPHYTES

The use of macrophytes (including angiosperms, macro-algae and bryophytes) as monitors of metals is potentially very useful but the adoption of this approach by statutory pollution-control authorities is limited, and it was not retained for the AETE field survey. Although several studies have used macrophytes to «monitor» the environment (see the case studies section), there have been few attempts to date to develop routine methods for practical application (Kelly 1988). A rapid survey of freshwater biological monitoring methods shows, on the other hand, the wide use of fish and aquatic invertebrates. Hellawell (1986) reported, however, that although the technique of using macrophytes to biomonitor contaminated environments is in its infancy and much more experience is necessary before it becomes possible to use it as a regulatory monitor, nonetheless one can confidently expect that the method will be adopted widely, especially for alerting authorities to intermittent discharges of metals.

Sortkjaer (1984) discusses some of the reasons why aquatic vascular plants are so rarely used as test organisms in ecotoxicological studies, even though we know much about their biology and life processes. He cited papers which suggest that submerged macrophytes could be used as indicators of the degree of pollution of running water. From an ecotoxicological point of view, these papers suggested that it would be possible to make a good prediction of field trends from experiments with macrophytes in laboratory aquaria; in fact, there was close agreement between laboratory results and those observed in the natural river system. Unfortunately, because these papers, as well as other interesting ones, are in German (e.g. Kohler 1974; Abo-Rady 1980; Melzer 1985), they have been largely ignored in North America.

This section of the technical evaluation reports a literature review concerning the use of macrophytes to monitor trace metal contamination, particularly contamination due to mine activities, in the freshwater aquatic environment, and provides a critical evaluation of these biomonitoring methods.

## 1.2 CONCEPTUAL MODEL OF METAL UPTAKE BY MACROPHYTES

Rooted submerged aquatic plants can potentially absorb metals both through their leaves, via the surrounding water, and through their roots, via the interstitial water (Denny 1980; see Fig. 1). It is now generally accepted that the major route of uptake of nutrients, such as phosphorus, is through the root system; however, the relative proportion of uptake by the roots and shoots depends upon the water:sediment available nutrient ratio (Carignan and Kalff 1980). For metals, theoretically, both routes can also be used. For species having well-developed root systems and totally submerged foliage, and considering the generally very low concentrations of metals in the overlying water compared to the high potential pool retained in the sediment (St-Cyr *et al*. 1994), one might expect the plant to extract metals, as for nutrients, mostly from the sediments with subsequent translocation to the upper parts. However, uptake by submerged foliage would be expected to become important when metal concentrations in the surrounding water are high and/or when metal concentrations in the sediment interstitial water are low (Denny 1980; Guilizzoni 1991; St-Cyr and Campbell 1997).

Some metals seem to be translocated easily in the plant, in both acropetal and basipetal directions (as for Cu: Welsh and Denny 1979, and Cd: Mayes *et al*. 1977; Brinkhuis *et al*. 1980), while other metals appear to be rather immobile (as Ni and Cr: St-Cyr and Campbell 1997, and Pb: Welsh and Denny 1979).

Free-floating plants extract their metals directly from the water column; however, they are mobile and therefore less suitable as biomonitor organisms than stationary rooted species (Crawford and Luoma 1993). Leaves of emergent (e.g. *Typha*) and floating-leaved plants (e.g. *Nuphar*) can be contaminated directly by atmospheric fall-out (Campbell *et al*. 1985; Crawford and Luoma 1993) and may be less reliable indicators if evaluation of the bioavailability of metals in sediment interstitial water/water column of the studied water bodies is the goal of the study.

Many researchers have attempted to relate metals in sediment with metal concentrations in plant tissues (see the case studies section). A literature review covering some 105 cases where metal concentrations had been determined both in aquatic plants and in the underlying sediment (Campbell *et al*. 1985) revealed that fully 65% of the cases showed no relationships between these two parameters; in an additional 5% of the cases, a negative correlation existed between the metal content of the plant and that of the sediment. A proposed possible explanation for this lack of relation is that researchers have tended to work with correlations between metals in plants and the **total** metal concentrations in sediment, rather than consider the implications of metal partitioning in order to estimate the bioavailable metal concentrations (Campbell *et al*. 1985). And some have worked with some types of partial extraction procedure performed on dried sediments (see the case studies section). The drying step may profoundly influence metal partitioning, and the results of a given extraction procedure performed after drying generally differ markedly from those obtained with the original fresh sediment. This is of particular importance for those metals originally present in the interstitial water or loosely held at adsorption sites (Campbell and Tessier 1988).

It is now generally accepted that total metal concentrations in sediment overestimate the actual bioavailable metal concentrations for benthic organisms, including rooted plants (Campbell and Lewis 1988; Adams *et al*. 1992), because a large part of the metal may be in unavailable form («lattice-bound» or «residual» metal). High total metal levels in sediment do not necessarily correspond to elevated plant tissue concentrations of these metals (Schierup and Larsen 1981; Miller *et al*. 1983). Given the inability of plants to take up particulate metals directly (the metal must first pass into solution before it can be taken up), a more logical approach would be to consider metal concentrations in sediment interstitial water. For aqueous exposures, the Free-Ion Activity Model predicts that the biological response will vary directly as a function of the free-metal ion concentration [$M^{Z+}$] (Campbell 1995). However, estimating free-metal ion concentration in the sediment interstitial water is not easy. Direct determination has rarely proved feasible, principally due to the relatively low metal concentrations involved, and the difficulty of obtaining sufficient sample volumes. However, using partial extraction procedures to partition metals in sediment among various defined forms, one can derive surrogate measures of the free-metal ion concentration in the interstitial water [$M^{Z+}$] (Campbell and Tessier 1996). This approach assumes that the free-metal ion concentration at the sediment-water interface is controlled by competitive sorption of the metal on the various sediment phases present in oxic sediments (Tessier 1992). The partitioning of metals in sediment (i.e. their distribution among different geochemical substrates) varies with each of the metals considered, for each metal has his own particularities and is influenced differently by environmental factors such as pH and Eh, the abundance of organic and inorganic materials and the concentrations of competing ions and solution ligands (Luoma 1989). The calculated values of [$Cd^{2+}$] successfully predict the Cd concentrations in soft tissues of the freshwater bivalve *Anodonta grandis* (Tessier *et al*. 1993), in the aquatic insect larva *Chaoborus punctipennis* (Hare and Tessier 1996) and in the tissues of *Vallisneria americana,* a submerged rooted angiosperm (St-Cyr and Campbell 1997); the calculated values of [$Pb^{2+}$] also successfully predict the Pb concentrations of *V. americana* tissues (St-Cyr and Campbell 1997; see the case studies section). The free-metal ion concentration in the interstitial water of the sediment would appear in the actual state of the research to be the more useful and more mechanistically meaningful measure to predict metal uptake by plants and other benthic organisms.

Metals found in above-ground parts of submerged plants can come from direct uptake from the surrounding water and/or from acropetal translocation from the roots in rooted species. Foliar metal contamination directly from the water column can be suspected to occur when there are high metal concentrations in the water column. In lakes, one normally expects to find a relation between [M] in water and [M] in sediment.

However, in flowing waters such as in the St. Lawrence River, high point source metal concentrations in the water are not necessarily reflected in the metal contamination of the sediment (St-Cyr and Campbell 1997). In some of the case studies depicted in the next section, metal concentrations in plant tissues are compared to metal concentrations in the ambient water. However, it is now appreciated that aqueous/dissolved metal concentrations reported before about 1990 were often erroneous, because of inadvertent contamination of the water sample (notably during the sample collection step; see Coale and Flegal 1989; Benoit 1994; Benoit *et al*. 1997). Admittedly, this may be less problematic in regions where gross contamination of the environment has occurred, and where metal concentrations are well above normal background levels (e.g., cases of acid mine drainage), but nevertheless the reader must be aware of possible erroneous values of dissolved metal concentrations in some of the case studies reported.

From this section about metal uptake by macrophytes, the reader must be aware that in several of the case studies reported in the next section, wrong evaluations of the bioavailable metal concentrations in the sediment as well as in the water were used to explain metal concentrations in aquatic plants. However, as most of these case studies are Canadian ones, showing the historic use of macrophytes as biomonitor organisms in environments affected by mining activities, they have to be included in the present report. It is hoped that further researches will take into consideration the present knowledge about environmental metal concentrations most likely to affect metal concentrations in plant tissues.

## 1.3 CASE STUDIES

### 1.3.1 Macrophytes and biomonitoring at mine sites

#### 1.3.1.1 Sudbury, Ontario

Sudbury is located in central Ontario, approximately 60 km northeast of Lake Huron, on the Canadian Shield. This area has known extensive logging of the forests since 1872, coupled with extensive mining and smelting operations since the mid-1880's (Amiro and Courtin 1981). In the vicinity of Sudbury, nickel and copper smelting at Copper Cliff, Coniston and Falconbridge has contributed large quantities of $SO_2$ and metals to the environment.

Gorham and Gordon (1963) performed a survey of the aquatic vegetation in ponds and lakes of the Sudbury area affected by the smelters. They reported that there was a reduction in the species diversity of submerged and floating macrophytes with decreasing distance from the smelters and as the concentration of sulphate in the water increased. Within two miles of the smelters, the total number of species observed ranged from 0 to 4; between 2 and 5 miles this range increased to between 2 and 8 species, while outside 15 miles the range was from 6 to 24. Ranges for pH were 3.3 to 6.1 within 2 miles and 4.4 to 7.4 outside 15 miles. Strong acidity was not accompanied by low Ca levels, as is often the case in acid bog lakes, but rather by higher concentrations owing to increased weathering of Ca from the soils by sulphuric acid (Gorham and Gordon 1963). Often only *Typha* spp. and *Phragmites australis* were sufficiently tolerant to survive close to the smelters.

The same phenomenon was reported for terrestrial plant communities influenced by pollution sources (Amiro and Courtin 1981; Gorham and Gordon 1960). Near the smelters, in very disturbed areas, first appeared a group of plants most of which were not common in the more mature forests of the region. These plants were enabled by their relative tolerance of sulphur dioxide to colonize the ground once competition from normal forest species is reduced owing to air pollution. Following this group of plants was a series of species which were able to exist both in the normal mature forest and in more or less heavily polluted situations. Finally, species of lesser tolerance were encountered, until the most sensitive species appeared (Gorham and Gordon 1960).

The list of submerged and floating leaf species most often encountered (species with emergent leaves were not included, unless growing wholly submerged), following a gradient of more to less contamination is (Gorham and Gordon 1963):

*Leptodictyum riparium* (a moss)

POLLUTED

*Eleocharis acicularis v. submersa*

*Juncus pelocarpus f. submersus*

↓

*Sagittaria*

*Eriocaulon septangulare*

*Isoetes muricata*

↓

*Nymphaea odorata*

*Lobelia dortmanna*

*Isoetes riparia*

↓

*Myriophyllum tenellum*

*Nuphar*

*Utricularia vulgaris*

LESS

*Potamogeton epihydrus v. nuttallii*

POLLUTED

Gorham and Gordon (1963) reported that *Leptodictyum riparium* and *Eleocharis acicularis v. submersa* seemed to be most tolerant of smelter pollution, being found within 2 miles of the smelters, while *Utricularia vulgaris* and *Potamogeton epihydrus v. nuttallii* appeared rather sensitive to it, being observed only beyond 15 miles from the smelters. Gorham and Gordon (1963) realized, however, that the sulphate itself was probably not the cause of the disappearance of the aquatic vegetation. They found ponds where the number of species was low even at pH above 6.0. Because sulphate ions were unlikely to be toxic at the levels encountered then, and since pollution also increased the concentrations of metals in the waters, most probably Cu and Ni, it was suspected by the authors that these metals may have reached toxic levels near the smelters. They concluded that it is quite conceivable that high acidity was of minor importance and that metal toxicity was the major factor limiting floristic variety in waters receiving fallout from the Sudbury smelters.

This was later confirmed by Yan *et al*. (1985). In central Ontario lakes with low metal concentrations, they observed no relationship between the richness of tracheophytes and pH of lake water (pH varying from 4.0 to 7.0); if lakes near Sudbury were included in the analysis, there was then a clear pattern of decreasing richness (species number) with increasing levels of Cu and Ni in the lake water (lakes having pH $\leq$ 5.3 and varying levels of Cu [1 to 360 µg/L] and Ni [2 to 3700 µg/L] in the water column). They concluded that in metal-contaminated acidic lakes, high concentrations of metals seemed to exert a toxic effect on macrophytes. Bryophyte richness increased with acidity, while no charophytes were found in lakes having a pH < 5.2. Among angiosperms, *Eriocaulon septangulare* and *Eleocharis acicularis* were the most frequently encountered species.

There was a major reduction of sulphur dioxide emissions in the Sudbury Basin during the 1970s, due primarily to improvements in the efficiency and pollution control of smelters in the area. Local deposition was also reduced considerably by the replacement of several shorter stacks with the Inco «super stack» in 1972 (Gunn 1995). Since sulphur dioxide emissions began to decline in 1970, changes have been documented in both land and lake environments and biota, with a rising in pH, declining sulphate ion concentrations and a concomitant increase in species richness of algae, invertebrates, and fish (Keller and Gunn 1995). These improvements were probably related to a decrease in metal concentrations in the water, including Cu and Ni.

Hutchinson *et al*. (1975) worked on three rivers flowing into Wanapitei Bay, located approximately 50 miles southwest of Sudbury. The Wanapitei River flows through the highly metal-contaminated areas of the Sudbury region, where acid soils increase the solubility of metals, particularly Ni and Cu, which are released into water bodies draining the contaminated areas. The French and Pickerel rivers drain uncontaminated areas. In the Wanapitei River near Wanapitei Bay, Ni concentrations in water reached 35-55 µg/L and Cu reached 8-15 µg/L, while in the uncontaminated rivers, concentrations reached 5-7 µg/L for Ni and 2-6 µg/L for Cu. Zinc was also measured as a control metal, in that it is not smelted at Sudbury.

The metal concentrations in aquatic macrophytes collected over the study area showed:

1) Species specificity for uptake;

2) A considerable concentration factor compared with levels in water, reaching 11400 for Ni, 9000 for Cu and 6600 for Zn in *Potamogeton* tissues;

3) A gradient in concentration among sites, paralleling that for both sediments and water.

Other conclusions were:

- Roots of *Equisetum palustre* were uniformly higher in all 3 metals (Cu, Ni, Zn) than were stems;

- The water lily *Nuphar variegatum* showed highest concentrations in its large floating leaves;
- Concentrations of Ni in the leaves of both *Potamogeton* sp. and *Anacharis* (*Elodea*?) were very high at sediment-contaminated sites.

Coefficients of correlation were calculated between metal levels in plant tissues and metal levels in surficial sediment. The metals in sediment represent the TOTAL metal concentrations, as extracted using hydrofluoric:nitric digestion (HF:$HNO_3$) (Hutchinson *et al*. 1975; Table 1.1). Significant correlations occurred between sediment Ni levels and levels in the roots of *Nymphaea* and between sediment Cu levels and levels in the roots of *Eleocharis* and *Nymphaea*. With ln transformations, significant correlations were also found between sediment Ni levels and concentrations in *Eleocharis* and *Equisetum* roots. Poor correlations existed between both Cu and Ni sediment levels and levels in *Nuphar* roots, and between Zn sediment levels and concentrations in roots of all plant species considered. It should be noted that unlike plants, animals (clams, crayfish, fish) exercised remarkable selectivity in metal uptake and excretion (Hutchinson *et al*. 1975).

Taylor and Crowder (1983a) collected one *Typha latifolia* sample in each of 28 wetlands along a 76-km transect extending northwest from Sudbury, following isopleths of aerial metal deposition. *Typha latifolia* inhabited all the contaminated wetlands visited, in which the pH of the soil-sediment varied from 3.6 to 5.6. Soil-sediment and plant parts were analyzed for Cu, Ni, Zn, Fe, Mn, Mg and Ca. Plants were subdivided into roots, rhizomes, leaves and reproductive tissues. DRIED soil-sediment samples from 15 cm-deep were analyzed using three different types of extractant: total, using $HNO_3 : H_2O_2$; ammonium acetate extractable metals, using $NH_4OAc$

**Table 1.1**. Correlation coefficients between the Ni, Cu and Zn concentrations (µg/g) in roots of plants sampled in the French River in 1974, and the total metal concentrations in surficial sediments (µg/g), with and without ln transformation of the data. From Hutchinson *et al*. (1975).

| Metal in plant tissues *vs*. | N | Ni in sediment | Cu in sediment | Zn in sediment |
|---|---|---|---|---|

| | | | with ln transformation | | with ln transformation | |
|---|---|---|---|---|---|---|
| *Eleocharis* roots | 11 | r=0.544 | r=0.685** | r=0.693** | r=0.654* | r=-0.100 |
| *Equisetum* roots | 17 | r=0.282 | r=0.487* | r= 0.004 | r=0.232 | r=-0.365 |
| *Nuphar* roots | 14 | r=0.212 | r=0.332 | r=-0.015 | r=0.090 | r=-0.062 |
| *Nymphaea* roots | 10 | r=0.652* | r=0.396 | r=0.841** | r=0.680* | r= 0.096 |

*P<0.05; **P<0.01

acidified to pH 4.2 with acetic acid; and DTPA-extractable metals. However, the extractants used to obtain «total» metal concentrations ($HNO_3$:$H_2O_2$) are not strong enough to dissolve all inorganic particles, and gave in fact a metal fraction ressembling the other two metal extractants. Total Cu and Ni in the soil-sediment material declined as distance from the smelters increased.

*Typha latifolia* roots showed higher concentrations of Ni and Cu than the rhizomes and aboveground tissues, with 13 to 265 µg/g Cu and from not detectable to 388 µg/g Ni. Also, concentrations of Cu and Ni in belowground and reproductive tissues were significantly correlated with soil-sediment metal concentrations; however concentrations in the leaves were not. No significant correlations were obtained with Zn (Table 1.2).

**Table 1.2**. Correlation coefficients between the Cu, Ni and Zn concentrations (µg/g) in belowground organs of *Typha latifolia* sampled in 1980 in wetlands located along a 76-km transect extending northwest from Sudbury, and the total soil/sediment metal concentrations (µg/g). From Taylor and Crowder (1983a).

| Metal in *Typha latifolia* *vs.* | Cu in soil-sediment | Ni in soil-sediment | Zn in soil-sediment |
|---|---|---|---|
| Roots | r=0.508* | r=0.373* | r=-0.180 |
| Rhizomes | r=0.684* | r=0.666* | r=-0.120 |

*P<0.05

Taylor and Crowder (1983a) concluded that despite high levels of Cu and Ni in the soil-sediment, these metals were largely excluded from the aboveground tissues of *Typha latifolia*, which had concentrations ressembling those typical of uncontaminated sites. However, some caution must be used when considering these conclusions:

1) Root material included both soil and water roots, and iron staining was visible on both roots and rhizomes. The authors reported that the elevated metal concentrations in roots may reflect both external contamination and internal immobilization of metallic ions. This can also lead to «false» significant correlations with soil-sediment metals, due to the presence of residual soil on roots and rhizomes.

2) The authors concluded that plants can exclude toxic levels of metals from the aboveground parts, particularly Cu, as shown by their low concentrations relative to soil-sediment and the lack of correlation with soil-sediment metals. *Typha latifolia* is reported in the literature to possess an inherent or constitutional tolerance to metals. Such a conclusion must be considered carefully, since the free-metal ion concentrations in the sediment, presumably representing the fraction bioavailable for the plant, were not measured, and the elevated metal concentrations in the soil-sediment could well be in a form unavailable for the plant, and not excluded by it. In fact, when *Typha latifolia* was grown in the laboratory in solution cultures containing these same metals, as Ni- and Cu-EDTA complexes, the plant was reported to be unable to minimize entry of Cu and Ni into leaf tissue, and accumulation of Cu and Ni by roots and leaves showed a significant linear relationship with concentrations of metals in the nutrient solution (Taylor and Crowder 1983b). However, even these last results are intriguing, since current thinking would indicate that neither Ni-EDTA nor Cu-EDTA should be available to the plant, unless an appreciable portion of the complexes dissociated under the conditions of the experiment. It would be interesting to re-analyze the results of Taylor and Crowder (1983b) using equilibrium calculations to determine the speciation of Ni and Cu in the solution cultures.

Within-site variation of metal uptake was studied at one sampling site where 25 shoots of *Typha latifolia* were collected and metals in the aboveground parts analyzed. The authors reported that within-site variance appeared to be small in comparison with between-site variance.

Reimer and Duthie (1993) collected *Eriocaulon septangulare*, *Nuphar variegatum*, *Nymphaea odorata* and *Pontederia cordata* in lakes from the Sudbury and Muskoka-Haliburton regions of Ontario, and investigated possible relationships between Zn and Cr levels in aquatic macrophytes and water and sediment variables. The four plant species studied showed high frequency in both contaminated and uncontaminated lakes. Collected plants were rinsed with lake water to remove residue and sediment, and separated into root/rhizome and shoot/leaf portions; *Eriocaulon septangulare*, due to its small size, was not separated into root and shoot portions and consequently all estimates for this species are whole plant values. Both plants and sediments were oven dried and TOTAL Zn and Cr concentrations were determined by neutron activation analysis. Although concentrations of Zn and Cr differed greatly among the four species studied, both metals were consistently higher in *Eriocaulon septangulare*. In plants where roots and shoots were analyzed separately, root and rhizome tissue contained higher Zn and Cr than shoot tissues of the same species and site. For *Eriocaulon septangulare*, sediment Zn was the best predictor of plant Zn ($r^2$ = 0.707), while sediment Cr and Ca were the best predictors of plant Cr ($r^2$ = 0.564). Chromium had been reported to possibly adsorb on the calcium carbonate which frequently covers the surface of aquatic plants, perhaps explaining the observed relationship between plant Cr and Ca. Reimer and Duthie (1993) reported that no significant trends were detected throughout the growing season in macrophyte concentrations of either metal. However, sampling was done from 29 May-9 June, 26 June-7 July and 31 July-11 August. To really study the possible seasonal variation in metal concentrations of plant tissues, samples should also be collected earlier in the spring (as soon as the shoot emerges) and late in autumn (when the plant is senescing).

Miller *et al*. (1983) studied the patterns of accumulation of Ni, Cu, Zn, Pb, Cd, Fe, Mn and Al in two species of higher aquatic plants (*Eriocaulon septangulare* and *Eleocharis acicularis*) and two kinds of bryophytes (*Fontinalis* spp. and *Sphagnum* spp.) found in soft-water central Ontario lakes (including Sudbury, Killarney, Parry Sound, Muskoka and Haliburton areas). TOTAL metal concentrations in dried sediment (from cores 10 cm deep) were determined after digestion with HCl:$HNO_3$. Collected plants were washed by hand until they were considered free from residue before analysis. The results showed that the partitioning of each metal between roots and shoots of higher aquatic plants varied with the metal and the overall level of metal enrichment of the site. Also, there was interspecific variation in metal accumulation, but the greatest differences were between vascular plants and bryophytes, rather than within these groups.

*Eriocaulon septangulare* was at first proposed by Miller *et al*. (1983) as a potential candidate for a biological indicator of metals in the environment. They reported that this plant is representative of the area, widely distributed, easy to collect and identify and shows both high tolerance to several metals and correspondingly high concentration factors. However, the authors were unable to link measured metal levels in plant tissue with metal levels in the sediment, and so concluded that metal accumulation in *Eriocaulon septangulare* had no predictive value as an indicator of environmental metal contamination. They thought the total metal concentration in the sediment to be representative of the amount available to the plants, in the absence of a suitable technique to measure metal availability. This led to ambiguous observations as a high level of environmental contamination does not necessarily correspond to elevated plant tissue concentrations of these metals (Miller *et al*. 1983).

#### 1.3.1.2 The Moira River system, Ontario

The Moira River drainage basin forms part of the eastern Lake Ontario drainage basin, and discharges into the Bay of Quinte of Lake Ontario. The area, centred around Deloro, Ontario, has been a centre for mining and smelting activities in eastern Ontario for over 100 years, producing Fe, Au, Cu, As, Co and Ni. Raw, as well as treated mining and smelting effluents, were discharged mainly into the Moira River. Mining and smelting ceased in the 1970s, but sediments in the Moira River system have remained contaminated.

Mudroch and Capobianco (1979) determined the concentrations of Ni, Co, Cu, Pb, Zn, Cd, Cr and As in aquatic sediments, water and macrophytes, collected along the Moira River (at Wolf Lake, Moira River, Moira Lake and Stoco Lake) contaminated by mine effluents. The TOTAL metal concentration in sediment samples was determined by digestion with a mixture of concentrated $HCl:HNO_3$ (1:1) for 90 min at 90°C. To determine the concentration of loosely bound metals, DRIED sediment samples were digested with 0.5N HCl for 16 h at room temperature. *Myriophyllum verticillatum*, *Elodea canadensis*, *Nymphaea odoratae* and *Pontederia cordata* were sampled in May, July and

August. *Myriophyllum verticillatum* and *Elodea canadensis* were collected with roots; only above-ground biomass was collected for the other species. The authors argued that since roots represented an extremely small portion of the biomass of the two submerged plants, it was unlikely that metal concentrations observed were due to inclusion of sediments on roots when comparing with the concentrations found in the above-ground biomass of the plants. In May, above-ground parts of *Iris*, *Typha* sp. and bull rush were also collected from a swamp at the Doloro old smelter. The plants were washed in lake and/or river water and digested for metal determination.

*Myriophyllum verticillatum* and *Elodea canadensis* (submerged rooted plants) accumulated much more metal in their tissues than did *Nymphaea odorata* (a floating plant) and *Pontederia cordata* (an emergent).

The concentrations of metals in water from all sampling stations in the Moira River system were found to be generally low. The concentrations of metals in *Myriophyllum verticillatum* and *Elodea canadensis* seemed to be more related to the concentrations of metals in sediments. Correlations were found between the concentrations of total Ni, Co and Cu in the bottom sediment and in the two submerged macrophytes; however, there was no significant correlation between the amounts of these metals extractable by 0.5N HCl from the sediments and the concentrations in the macrophytes. No coefficients of correlation or scattergrams can be found in the paper of Mudroch and Capobianco (1979).

The concentrations of metals in the tissues of submerged plants decreased from May to August; however, it was observed that the amount of biomass considerably increased during this period. The diluting effect of a large increase in the plant biomass was probably the cause of these declining concentrations (Mudroch and Capobianco 1979).

The Bay of Quinte receives drainage from the Moira River which carried sediment from mines into the Bay from 1880s to the 1960s. Crowder *et al*. (1989) sampled sediment, macrophytes and snails in wetlands close to the Moira River and at Hay Bay (part of the Bay of Quinte presumably unaffected by mine effluents) located 20 km from the Moira River input. TOTAL metals in sediment (cores 10 cm in depth) and biota were determined by neutron activation analysis or by acid dissolution (using $HNO_3$, $HClO_4$, HF and HCl for sediment digestion).

Levels of As in *Myriophyllum spicatum* and *Vallisneria americana*, the two submerged plants studied, were higher close to the Moira River. This trend was consistent with differences in sediment As values and led the authors to suggest that both species are reliable monitors of such contamination on a regional basis. However, differences in accumulation between macrophyte species were also found; *Myriophyllum spicatum* acted as an accumulator of Pb (up to 9.6 ppm) whereas Pb in *Vallisneria americana* at the same sites was undetectable (Crowder *et al*. 1989).

#### 1.3.1.3 Rouyn-Noranda, Québec

Rouyn-Noranda is located in northwestern Québec, some 500 km northwest of Montréal, and is a major Cu/Zn mining and smelting complex. Tessier *et al*. (1982) carried out a survey of some lakes downstream from the Rouyn-Noranda area (Lake Beauchastel, La Bruère, Routhier, Pelletier and Montbeillard) and recorded the plant species most frequently encountered:

| | |
|---|---|
| *Eleocharis acicularis* | *Potamogeton richardsonii* |
| *Eleocharis smallii* | *Sagittaria latifolia* |
| *Glyceria borealis* | *Sparganium americanum* |
| *Nuphar variegatum* | *Vallisneria americana* |

Campbell *et al*. (1985) studied the relationships between the partitioning of Cu and Zn in lake sediments and their availability to *Nuphar variegatum*. It was supposed, for the lakes studied, that there was a spatial gradient in metal concentrations in the sediments, but not in the overlying water column. Collected plants were carefully washed and separated into stems and rhizome; a section corresponding to the previous year's growth was removed from the rhizome and saved (the remainder of the rhizome was discarded). The leaves also were discarded because of possible contamination from atmospheric deposition of metals directly on the exposed leaf surface. Sediments (0-2 cm strata; <850 µm) were subjected wet to a sequential extraction leaching procedure (Tessier *et al*. 1979) to determine the partitioning of Cu and Zn among various operationally defined geochemical fractions:

FRACTION 1 (F1): exchangeable metals. The sediment sample was extracted for 10 min with 0.5 mol/L $MgCl_2$ at pH 7.0;

FRACTION 2 (F2): metals bound to carbonates or specifically adsorbed. The residue from F1 was leached for 5 h with 1 mol/L NaOAc adjusted to pH 5.0 with HOAc;

FRACTION 3 (F3): metals bound to Fe-Mn oxides. The residue from F2 was extracted for 6 h at 95°C with 0.04 mol/L $NH_2OH\bullet HCl$ in 25% (v/v) HOAc;

FRACTION 4 (F4): metals bound to organic matter and sulfides. The residue from F3 was extracted at 85°C for 5 h with 30% $H_2O_2$ adjusted to pH 2.0 with $HNO_3$ and then at room temperature with 3.2 mol/L $NH_4OAc$ in 20% (v/v) $HNO_3$;

FRACTION 5 (F5): residual metals. The residue from F4 was digested with a 5:1 mixture of $HF:HClO_4$.

TOTAL: A separate subsample of sediment was analysed for total metals using the same reagents as used for determining Fraction 5.

Compared with the total metal concentrations in the lake sediments, the levels found in the plant tissue were low. The distribution of metals within the plant, among different organs, and even inside an individual organ (rhizome), was not homogeneous; metal concentrations in the stem generally exceeded those in the rhizome, but the ratios of $[M_{stem}]:[M_{rhizome}]$ varied considerably.

Statistical analysis was used to study the relationships between metals in the sediments and in plants. Campbell *et al*. (1985) proposed the following conceptual model. The sediment-bound metal is partitioned among the five operationally defined fractions, one or more of which may be in equilibrium with $M_i$, the metal in the interstitial water. Some fraction of $M_i$, depending on the environmental conditions prevailing in the interstitial water (e.g. [ligands], pH), exchanges with the metal bound at the rhizome surface. After transport into the plant, the metal enters the dissolved intracellular pool, from which it may be removed and stored within the rhizome itself or translocated towards the stem and eventually stored there. This scheme neglects possible metal uptake from the water column into the submerged plant parts.

No statistically significant correlations were observed between the Cu content of the rhizomes [$Cu_r$] and the total Cu concentration in the sediment. However, if the partitioning of Cu concentration in the sediment was considered, several statistically significant correlations did appear, notably between [$Cu_r$] and the concentrations of Cu in the fractions most readily extracted from the sediment (Table 1.3). Normalization of these concentrations with respect to the Fe content of the sediment improved the relationship with [$Cu_r$] suggesting that Fe may play a protective role in regulating Cu bioavailability (Table 1.4). The significant relation between the Cu content of the stem [$Cu_s$] and [$Cu_r$] is consistent with the hypothesis of a translocation pathway from rhizome to stem.

**Table 1.3**. Correlation coefficients between the metal concentrations (µg/g) in *Nuphar variegatum* sampled in 1981 in lakes of the Rouyn-Noranda area and the metal concentrations (µg/g) in the adjacent lake sediments subjected to a sequential extraction leaching procedure as described in the text. N=13 except for Cu(F1) for which N=10. From Campbell *et al*. (1985).

| | | Sediment fraction | | | | | |
|---|---|---|---|---|---|---|---|
| Metal | *Nuphar variegatum* | M(F1) | M(F2) | M(F3) | M(F4) | M(F5) | Total |
| Cu | Rhizome | r= 0.55* | r= 0.49* | r= 0.01 | r= 0.40 | r= 0.48* | r= 0.45 |
| | Stem | r= 0.12 | r= 0.22 | r= 0.85** | r= 0.15 | r= 0.55* | r= 0.26 |
| Zn | Rhizome | r=-0.31 | r=-0.37 | r=-0.31 | r=-0.31 | r=-0.35 | r=-0.34 |
| | Stem | r= 0.81** | r= 0.84** | r= 0.89** | r= 0.90** | r= 0.90** | r= 0.91** |

*P<0.05, **P<0.01

**Table 1.4**. Correlation coefficients between the metal concentrations (µg/g) in rhizomes of *Nuphar variegatum* and the metal concentrations (µg/g) in sediment fractions M(F1) and M(F2); effect of normalization with respect to Fe. From Campbell *et al*. (1985).

| | | Sediment fraction | | | | | |
|---|---|---|---|---|---|---|---|
| Metal | *Nuphar variegatum* | M(F1) | M(F1)/Fe(F1) | M(F1)/Fe(F3) | M(F2) | M(F2)/Fe(F2) | M(F2)/Fe(F3) |
| Cu | rhizome | r= 0.55* | r= 0.94** | r= 0.82** | r= 0.49* | r= 0.17 | r= 0.58* |
| Zn | rhizome | r=-0.31 | r= 0.33 | r=-0.35 | r=-0.37 | r=-0.31 | r=-0.35 |

*P<0.05, **P<0.01

In contrast with the results for Cu, no relationship could be discerned between the Zn content of the rhizomes and any of the Zn fractions in the sediment (Table 1.3), and there was a lack of correlation between the Zn concentration in the rhizomes and that of the stems. However, numerous correlations were observed between the Zn content of the stems and each of the Zn fractions in the sediment (Table 1.3). This led the authors to speculate that (i) *Nuphar variegatum* accumulates Zn directly from the water column and (ii) there exists an exchange mechanism between the interstitial water in the sediments and the overlying water column.

Thus, the statistical analysis suggested that a significant portion of the Cu burden in *Nuphar variegatum* was probably derived from the sediments, whereas the Zn content of the plant originated largely in the water column (Campbell *et al*. 1985).

Within-site variability in trace metal content of *Nuphar variegatum* organs was estimated at two sites by Campbell *et al*. (1985) and show an appreciable inter-plant variability at both sites: the relative standard deviations observed for Cu (20-40%) and Zn (5-50%) were considerably greater than the variability attributable to the analytical procedure itself (±5-10%, as determined by replicate analyses). Possible explanations suggested by the authors include the existence of distinct microhabitats within the sampling site, the collection of specimens of differing age and the sampling of plants at slightly different stages in their growth cycle.

Metal ions in the rhizomes could be present as a result of three factors: i) redistribution along the phloem as carbon reserves are build up in the rhizomes, ii) limited uptake of ions by roots arising at the nodes in the rhizomes and iii) external contamination remaining after washing, e.g. in the form of iron deposits (Taylor and Crowder 1983a). Compared to rhizomes, analysis of root tissues should better reflect what is actually taken up by the plant.

Campbell and Tessier (1989) reported additional regression analyses used to examine the relationships between metal concentrations in other plant species underground organs (roots/ rhizome) from the Rouyn-Noranda area and the concentration of metals extracted from the most readily extracted fractions from the sediment, normalized with respect to the Fe content of the sediment (Table 1.5); this ratio is thought to represent a surrogate measure for [$M^{Z+}$], the free- metal ion concentration in the interstitial water, at the oxic sediment/water interface. The usefulness of this ratio as a predictor of the sediment metal bioavailability for rooted macrophytes varied among metals (Cu > Pb, Zn) and among plants (*Potamogeton richardsonii* > *Eleocharis smallii*, *Nuphar variegatum*, *Sparganium americanum* > *Glyceria borealis*). Campbell and Tessier (1989) suggested that *Potamogeton richardsonii* may prove useful as a biomonitor species for sediment-associated metals.

**Table 1.5**. Correlation coefficients between the metal concentrations (µg/g) in belowground parts of rooted aquatic plants and the metal concentrations (µg/g) in M(F3) normalized with respect to Fe. From Campbell and Tessier (1989).

| Plant species | sediment [M](F3)/[Fe](F3)[a] | | |
|---|---|---|---|
| roots/rhizome | Copper | Lead | Zinc |
| *Potamogeton richardsonii* | r= 0.85** | r= 0.79** | r= 0.94** |
| *Sparganium americanum* | r= 0.92** | r= 0.14 | r= 0.91** |
| *Nuphar variegatum* | r= 0.74** | r= 0.52 | r=-0.30 |
| *Eleocharis smallii* | r= 0.81* | r= 0.80* | r= 0.01 |
| *Glyceria borealis* | r=-0.02 | r=-0.13 | r=-0.11 |

*P<0.05, **P<0.01

[a] Metal (Cu, Pb, Zn) in sediment fraction F3, as defined in the text, divided by the Fe concentration in the same extract. Provided the sediment pH is reasonably constant, this ratio represents a surrogate measure of the free-metal ion concentration at the sediment-water interface (Campbell and Tessier 1996).

#### 1.3.1.4 The Northwest Miramichi and Tomogonops River system, New Brunswick

Besch and Roberts-Pichette (1970) studied the copper-zinc mining pollution effects on riparian and aquatic vascular plants in the Northwest Miramichi and Tomogonops River system, of northern New Brunswick. A rough index of pollution severity along the river was devised based on the absence of species or species groups which would normally be present under unpolluted conditions. In the unpolluted parts of the Tomogonops River, not affected by mines, rich colonies of *Ranunculus trichophyllus* and *Potamogeton* sp., as well as submerged mosses were characteristically present.

Alongside the river, there were belts of gravel which were periodically inundated; at the sites closest to the mine where the river water could contain up to 12 mg/L Cu and 65 mg/L Zn, they were barren. As the metal concentrations decreased, the plant cover increased (the amount of pollution decreased with downstream dilution). In regions with medium to high pollution, there was a sparse cover of *Equisetum arvense*, *Typha latifolia*, Cyperaceae and Graminaceae. *Equisetum arvense* has a wide ecological range, and did not appear to be as affected as other plant species by the mining operations. Further downstream, where the pollution was lower, dicotyledons returned and only the submerged vascular plants were eliminated.

It should be noted that near the mining site, there were enormous fluctuations in streamflow and the substrata were very unstable; permanently submerged vascular plants did not establish themselves in the surveyed stretches where these conditions prevailed. Further downstream, the elimination of submerged plants was the main visible pollution effect. Besch and Roberts-Pichette (1970) concluded that «the absence of these plants from streams which would normally support them can therefore be regarded as a very sensitive indication of the presence of toxic concentrations of heavy metals. The absence of certain riparian species which are widely distributed in the area and are usually of high abundance and fidelity along the river banks can (also) be indicative of heavy metal pollution (e.g. *Cicuta bulbifera*, *Cardamine pensylvanica*, *Sium suave* and *Salix interior*).» However, considering, as mentionned in section 1.2, the difficulty of obtaining reliable dissolved metal concentrations in the water column, that no measurements of metal concentrations in the soil/sediment were taken, that other factors (e.g. pH, turbidity, water depth, current velocity) vary along river reaches (Vannote *et al*. 1980) influencing the establishment of submerged plants, and that, as discussed in section 1.4.1 and observed by the authors, harsh conditions, other than high metal concentrations, prevailed in environments near mining activities (e.g. rough substrate, few organic matter deposits, irregular water levels, poor nutrient (N, P) levels, high turbidity and suspended solids), it would be more realistic to conclude that the absence of plants may be due to the general «pollution» created by mining operations, and may be not only, or not at all, due to metal concentrations in the water column.

#### 1.3.1.5 The Nepisiguit River system, New Brunswick

The purpose of the study of Ray and White (1976) in the Nepisiguit River system of northeastern New Brunswick, where intensive base-metal mining operations had substantially deteriorated the water quality, was to evaluate the freshwater plants as biological monitors of metal pollution. The Cu, Zn, Cd and Pb concentrations of two vascular plants, *Potamogeton richardsonii* and *Equisetum fluviatile* forma *linnaearum* and of a blue algae *Oscillatoria* are reported as well as their possible use as indicator species.

Plants were collected in September from two widely different locations (three sampling sites in each), transferred to the laboratory in frozen conditions, then thawed and thoroughly washed with deionized water. The two vascular plants were separated into below-ground (rhizomes and roots) and above-ground (leaves and stem) parts. Plant parts were then digested and analysed along with water samples at all sampling sites for Cu, Zn, Pb and Cd. The metal levels in the water samples only indicate the quality prevailing at the time of sampling and not average levels over a long-period term.

None of the species inhabited all the collection sites or even a majority of the locations. The alga *Oscillatoria* was found only in rather polluted streams, whereas the vascular plants were not found in these sites. *Oscillatoria* from both sampling stations showed relatively constant levels of Cd and Zn, but the Cu and Pb levels differed widely and led the authors to speculate that the alga selectively accumulates Pb and might be an effective indicator organism for Pb pollution (Table 1.6).

**Table 1.6**. Metal concentrations (µg/g) in aquatic plants sampled at six sampling sites in the Nepisiguit River system. From Ray and White (1976).

| Site # | Plant sp. | Plant part | Cu (µg/g) | Zn (µg/g) | Cd (µg/g) | Pb (µg/g) |
|---|---|---|---|---|---|---|
| 4 | *Oscillatoria* | | 70 | 500 | 0.9 | 25 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 5 | *Oscillatoria* | | 240 | 480 | 1.0 | 570 |
| 1(u) | *Potamogeton* | Below-ground | 8 | 100 | 1.3 | 3.7 |
| | *richardsonii* | Above-ground | 3 | 110 | 0.7 | 2.5 |
| 3 | *Potamogeton* | Below-ground | 200 | 1800 | 6.7 | 13 |
| | *richardsonii* | Above-ground | 170 | 2900 | 4.9 | 4.8 |
| 2 | *Equisetum* | Below-ground | 180 | 1000 | 5.5 | 1.4 |
| | *fluviatile* | Above-ground | 30 | 900 | 6.1 | 1.2 |
| 6 | *Equisetum* | Below-ground | 110 | 6000 | 3.6 | 47 |
| | *fluviatile* | Above-ground | 10 | 1400 | 0.9 | 5.6 |

(u) uncontaminated, above mining sites.

In the vascular plants, the roots in general showed much higher levels of metal content, except in above-ground parts of *Potamogeton richardsonii* where Zn levels were high. This same plant collected at an uncontaminated site, above any mining activities, had relatively low levels for all metals and reflected only the geochemistry of the area, whereas the other sample collected further downstream showed quite high values for all elements, reflecting the water quality of the receiving stream. The two samples of *Equisetum fluviatilis* were both collected in polluted waters, however the relative levels of pollution were qualitatively well reflected in the metal concentration of the samples (Table 1.6). The authors concluded the three species were useful as biological monitors to qualitatively reflect the long term water quality. However, considering the few sites in which each vascular plant species was effectively growing, N is very small and thus the conclusion must be considered cautiously.

Later, Ray and White (1979) reported the use of *Equisetum arvense* as a suitable biological monitor for metal pollution in the Nepisiguit River system. The plant specimens were collected from below the high-water line of contaminated rivers. This species grows at the edge of the water with shoots and reproductive structures usually independent of the water medium. The plants had sparse hair-like roots but prominent underground rhizomes which were in constant contact with the water-saturated substrate. Methods of analysis were as previously described.

The rhizomes and roots had higher metal contents than aerial stems in all cases, for samples collected in September (Table 1.7). The concentrations of Cu and Zn in the tissues qualitatively reflected the metal concentrations at the sampling sites. There was also a definite trend to high Cd levels in the plants from polluted sites, however, this was not as pronounced as in the case of Cu and Zn.

*Equisetum arvense* was reported to be found extensively beside waters affected by acid mine drainage and also in locations considered unpolluted. The pH of water at these locations varied from 3.8 to 7.5. Metal contents of the plants from the two uncontaminated sites (#4 and 5) possibly represent natural background levels of the area (Table 1.7).

The exact relationship between the concentrations of metals in the water and plant tissues was not established, owing to temporal variations in the water at the sampling sites.

**Table 1.7**. Metal concentrations in water (µg/mL) and in *Equisetum arvense* above- and below-ground parts (µg/g), collected at five sampling sites in the Nepisiguit River system in September 1976. From Ray and White (1979).

| Site # | Sample | Plant part | Cu | Zn | Cd | Pb |
|---|---|---|---|---|---|---|
| 1 | water | | 0.043 | 0.339 | <0.0005 | <0.005 |
| | plant | above-ground | 27 | 551 | 0.5 | 2.1 |
| | | below-ground | 181 | 841 | 3.0 | 3.4 |
| 2 | water | | 0.014 | 0.081 | <0.0005 | <0.005 |
| | plant | above-ground | 74 | 1017 | 2.4 | 3.4 |
| | | below-ground | 348 | 1618 | 7.3 | 7.3 |
| 3 | water | | 0.012 | 0.090 | <0.0005 | <0.005 |
| | plant | above-ground | 17 | 282 | 0.5 | 2.1 |
| | | below-ground | 43 | 331 | 1.0 | 6.0 |
| 4(u) | water | | 0.001 | 0.001 | <0.0005 | <0.005 |
| | plant | above-ground | 3 | 30 | 0.1 | 2.2 |
| | | below-ground | 11 | 65 | 0.3 | 3.1 |
| 5(u) | water | | 0.001 | 0.001 | <0.0005 | <0.005 |
| | plant | above-ground | 6 | 33 | 0.1 | 1.9 |
| | | below-ground | 9 | 41 | 0.3 | 2.0 |

(u) uncontaminated sites

Seasonal variations in the metal content of *Equisetum arvense*, collected in July and September, revealed that in general the metal concentrations were higher in July, when plants were growing, than in September (Table 1.8; Ray and White 1979).

The authors thought it reasonable to conclude that the differences in metal content of the tissues arise from environmental differences and do reflect the integrated metal concentrations in water over a long period. However, in using *Equisetum arvense* as a biological indicator, seasonal variations in metal concentrations in the tissues should also be taken into consideration in order to draw an effective conclusion.

**Table 1.8**. Metal concentrations in water (µg/mL) and in *Equisetum arvense* above- and below-ground parts (µg/g), collected at two sampling sites in the Nepisiguit River system, in July and September 1976. From Ray and White (1979).

| Site # | Sample | Part | Cu | | Zn | | Cd | | Pb | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | July | Sept. | July | Sept. | July | Sept. | July | Sept. |
| 3 | water | | 0.014 | 0.012 | 0.093 | 0.090 | <.0005 | <.0005 | <0.005 | <0.005 |
| | plant | above | 17.0 | 17.0 | 258.0 | 282.0 | 0.2 | 0.5 | 0.9 | 2.1 |
| | | below | 176.0 | 43.0 | 697.0 | 331.0 | 2.2 | 1.0 | 18.2 | 6.0 |
| 5 | water | | 0.001 | 0.001 | 0.001 | 0.001 | <.0005 | <.0005 | <0.005 | <0.005 |
| | plant | above | 10.0 | 6.0 | 34.0 | 33.0 | 0.1 | 0.1 | 1.3 | 1.9 |
| | | below | 26.0 | 9.0 | 43.0 | 41.0 | 0.3 | 0.3 | 4.7 | 2.0 |

#### 1.3.1.6 Flin Flon, Manitoba

Franzin and McFarlane (1980) collected five species of aquatic plants, *Utricularia vulgaris*, *Sparganium* sp., *Myriophyllum exalbescens*, *Nuphar variegatum* and *Calla palustris*, in the vicinity of a base metal smelter located at Flin Flon, Manitoba. Zinc was the major metal found in the fallout, but Cu, Cd, Pb and As in the fallout also originated from the smelter. The purpose of the study was to select an aquatic plant species suitable to be a metal pollution indicator.

Metal concentrations in the five plant species were determined from a contaminated (Lake 6) and a relatively uncontaminated (Thompson Lake) lake in 1975. Only leaves and stems of submergent plants or submerged portions of the plants were collected. Plant samples were cleaned as well as possible in lake water, then in tap water and rinsed twice with distilled water. DRIED sediment samples were treated with aqua regia ($HNO_3$:HCl, 1:3). Sediment and plant digests were analysed for Fe, Mn, Pb, Cu, Cd and Zn by flame atomic absorption spectrophotometry. Total As in sediment and plant samples was investigated separately.

Zinc provided the best indication of the presence of smelter fallout contamination in lakes but a summation of the concentrations of all the smelter-related metals in the plant tissues was used for an integrated evaluation of the plants as potential indicator species. As an indicator species, a plant should have a high metal tolerance and a high concentration factor; this criterion plus others such as the plant should be representative of the area, ubiquitous and easily collected, and easily and unequivocally identified, led to the selection of *Myriophyllum exalbescens* as the indicator species in the Flin Flon area (Franzin and McFarlane 1980). It was apparent that the surface area to volume relationship of these aquatic plants was an important determinant of metal uptake. Fleshy, heavily rooted plants such as *Nuphar* and *Calla* had about one fifth the metal concentrations in submerged leaves and stems as the ribbon-leaved or profusely-foliated plants such as *Sparganium*, *Utricularia* and *Myriophyllum* (Table 1.9).

**Table 1.9**. Metal concentrations (μg/g) in aquatic plants sampled from two Flin Flon area lakes (Lake 6 → contaminated and Thompson Lake → relatively uncontaminated) in 1975. From Franzin and McFarlane (1980).

| Lake | Plant sp. | Metals (μg/g dry weight) | | | | |
|---|---|---|---|---|---|---|
| | | Zn | Cu | Cd | Pb | As |
| Lake 6 | *Sparganium* sp. | 1460 | 63 | 5.0 | 27.0 | 25.0 |
| Thompson | | 54 | 38 | <0.2 | <0.9 | - |
| Lake 6 | *Utricularia vulgaris* | 1330 | 36 | 14.0 | 17.0 | 20.0 |
| Thompson | | 134 | 20 | 0.7 | <0.6 | - |
| Lake 6 | *Myriophyllum exalbescens* | 1640 | 66 | 6.0 | 27.0 | 40.0 |
| Thompson | | 78 | 32 | <0.1 | <0.6 | - |
| Lake 6 | *Nuphar variegatum* | 274 | 7 | 0.6 | 2.0 | 6.0 |
| Thompson | | 11 | 4 | <0.1 | <0.6 | 0.9 |
| Lake 6 | *Calla palustris* | 389 | 9 | 4.0 | 2.0 | 5.0 |

| | | | | | |
|---|---|---|---|---|---|
| Thompson | 190 | 11 | <0.1 | <0.6 | 1.0 |

The suitability of the chosen species was tested in 1976 by analysing its tissue metal concentrations in six lakes (Hamell, Cliff, Hook, Nesootao, Thompson and Lake 6) showing a range in metal contamination of water and sediment. Comparison of metal content of submerged portions of *M. exalbescens* samples collected in 1975 and 1976 in Lake 6 and Thompson Lake showed that there were large inter-year differences in metal concentrations (Lake 6: 1640 µg/g of Zn in 1975 *vs*. 12300 µg/g in 1976 and Thompson Lake: 78 µg/g of Zn in 1975 *vs*. 185 µg/g in 1976), probably related to a nearly two-fold difference in rainfall during the growing periods of the two years. Increased rainfall in 1976 was thought to have increased movement of metals from drainage basins into the lakes and consequently the metal concentrations in aquatic plants were higher. Comparison of *Myriophyllum exalbescens* tissue metal concentrations with the metal concentrations in lake waters and sediments showed that a fair correlation existed only for Zn in water. These results suggested that this plant may qualitatively indicate the degree of Zn contamination of aquatic environments if the contamination is severe. However, the authors concluded that analyses of aquatic plants cannot be relied upon to distinguish grades of metal contamination in their habitats which can be seen in water, sediment and fallout analyses.

Analyzing their data further, Franzin and McFarlane (1980) found that the Ca concentration of lake waters may have a modifying role on plant uptake of all metals, whether essential or non-essential for survival and growth. For example, plants from Nesootao Lake, a relatively uncontaminated lake with a Ca concentration of 10 mg/L, showed high metal concentrations (1470 µg/g of Zn, as high as the most contaminated lakes) whereas plants from Hook Lake, a highly contaminated lake with a Ca concentration of 39 mg/L, showed lower metal concentrations (848 µg/g of Zn).

#### 1.3.1.7 Other mine sites

*The Clark Fork River, Montana, USA*

Johns (1993) measured metal concentrations (Cu, Zn, Pb, Mn and Fe) in roots and leaves of *Typha latifolia*, collected from small riparian wetlands along the upper Clark Fork River, in Montana, USA. The headwater region of this river has been contaminated with metals from mining and smelting of copper-bearing ores. *Typha latifolia* was the dominant species of emergent macrophyte of the small riparian wetland areas along the river.

Plants, including shoot, rhizomes and roots, were sampled at 5 contaminated sites and one uncontaminated site, located in the Blackfoot River, a tributary of the Clark Fork. Metal concentrations in sediments, in both «near total sediment digestion» and 0.5N HCl extracts were determined, in both sieved (< 60 µm) and unsieved samples.

Metal concentrations in cattail roots and in both sediment fractions varied widely among sites. All contaminated sites showed elevated metal concentrations compared to the uncontaminated site. Metal concentrations were much more elevated in roots than in leaves of *Typha latifolia*. Significant positive correlations found between Cu and Zn in sediments and roots varied little whether sediment metal concentrations were based on «total» digests or HCl extracts. Use of sieved sediments resulted in only slight increases in coefficients of determination.

In an earlier study, Johns (1987) had studied the accumulation and partitioning of As in *Typha latifolia* and *Carex rostrata* in a reservoir contaminated by metal-enriched sediment (As, Cu, Zn, Pb) from upstream mining and smelting operations on the Clark Fork River, and at an uncontaminated site. Plants were separated into roots, rhizomes and leaves. All samples were carefully rinsed with distilled water. Roots and rhizomes were brushed gently with a toothbrush to remove all particulate material.

Arsenic accumulated to significantly greater concentrations in both above- and below-ground tissues of plants from contaminated sites, when compared with plants from the uncontaminated site. In both species, As concentrations decreased in the order roots >> rhizomes > leaves (for *Typha latifolia*, ranges of As concentrations obtained at the contaminated reservoir were roots 26-88 µg/g > rhizomes 3.8-61 µg/g > leaves 0.2-2.8 µg/g, and for *Carex rostrata* roots 34-377 µg/g > rhizomes 7.4-27 µg/g > leaves 0.7-4.8

μg/g). In the reservoir, total As concentrations in surface sediment ranged from 13 to 126 μg/g.

In both studies (Johns 1987, 1993), the author observed iron oxide precipitation on the roots and rhizomes of the plants and hypothesized that the high As concentrations found in below-ground tissues may be associated with these iron oxide root coatings. If As is predominantly associated with these coatings (As is known to be scavenged by and precipitated with iron hydroxides), rather than with internal tissues, then roots may exhibit greater As concentrations than rhizomes due to different surface to volume ratios.

*River Kokemäenjoki, Finland*

Aulio (1980) tested the ability of *Nuphar lutea* to accumulate Cu in the River Kokemäenjoki in western Finland, situated near a base metal (Cu) purification and processing plant. The surface layer (0-5 cm) of the substrate was sampled, oven-DRIED, and analyzed i) for total Cu concentrations (acid-extracted using 16N $HNO_3$ boiling to dryness twice, and HCl) and ii) for exchangeable Cu (using 0.5N HCl). Collected plants were washed with tap water, and separated into flower, petiole, leave and rhizome.

With increasing distance from the processing plant, the levels of Cu accumulated in sediments decreased sharply. Similarly, the levels of Cu in *Nuphar lutea* organs were markedly elevated at sampling sites near the metal processing plant, and decreased sharply with increasing distance above and below the waste discharge point. The highest Cu concentrations recorded for leaves and petioles were 98 μg/g and 115 μg/g, respectively. In contrast, the concentrations of Cu in the rhizomes were surprisingly low.

Correlations were calculated between the Cu concentrations accumulated in various plant organs and the levels of total and exchangeable proportions of Cu in the substrate.

The accumulation of Cu in petioles, leaves and rhizomes of *Nuphar lutea* was clearly proportional to the Cu levels in the corresponding surface sediments (Table 1.10).

**Table 1.10**. Correlation coefficients between the Cu concentrations (µg/g) in *Nuphar lutea* organs sampled in 1977 in the River Kokemäenjoki and the Cu concentrations in sediments (µg/g). N=7 sampling sites. From Aulio (1980).

| *Nuphar lutea* organs | Cu total | Cu exchangeable |
| --- | --- | --- |
| [illegible] | | |

rhizomes

flowers**P<0.01, ***P<0.001

Aulio (1980) reported that the fact that *Nuphar lutea* was able to survive and grow without any visible injuries in the habitats containing very high levels of Cu seemed to indicate well-developed tolerance to metal pollution, and showed the usefulness of this plant as a biological indicator of aquatic pollution.

Above-ground parts (leaves and shoots) of 15 plant species representing different life forms (emergent, floating-leaved and constantly submerged species) were also collected from a single sampling area in the Kokemäenjoki River estuary and were analysed for Cu, Zn, Mn and Fe (Aulio 1986). The differences in the metal concentrations between species could then be attributed to species-specific characteristics rather than to variations in environmental conditions. Exchangeable fractions of metals in the DRIED sediment were extracted with 0.5N HCl. In general, the submerged taxa had the highest metal concentrations, so they appeared to be the most efficient metal accumulators, while the floating-leaved and especially the emergent plants showed weaker metal accumulation.

The metal levels in the plant tissues of the six emergent macrophytes sampled were weakly correlated with the corresponding metal concentrations of the plant substrata (which is not surprising since the plants were collected from ONE particular sampling area; there was no gradient). Manganese was the only element showing a significant positive correlation between the plant tissues and sediments; the other metals showed non-significant (Fe) or even negative (Zn, Cu) correlations.

Of the plant species studied, *Potamogeton perfoliatus* and *P. obtusifolius* proved to be the most promising candidates for biological monitoring of metal pollution in aquatic environments. Among the emergent species, *Butomus umbellatus* was reported to be the most promising candidate as a selective accumulator of Fe, as for *Equisetum fluviatile*, and *Typha latifolia* was reported to be a selective accumulator of Zn. The floating-leaved taxa showed an «intermediate» accumulation capacity and thus appeared to the author to be of limited value as monitoring organisms (which is in contrast with the conclusion reported before in Aulio (1980)).

Furthermore, comparing shoot metal content of submerged plant species having different growth form, Jackson and Kalff (1993) found that understory species (e.g. *Vallisneria americana*) showed higher metal contents for a given sediment metal concentration than canopy-forming species (e.g. *Myriophyllum spicatum*) when they are growing together.

*Lakes Ullswater and Coniston Water, England*

The lakes Ullswater and Coniston Water, England, have been the sites of mining of metal ores from about 1700 until the present century. Several mines around the head of Ullswater were worked for Pb and subsidiary Zn ores; the largest was closed in 1962. Production stopped at the Coniston copper mines in 1915. Seepage water from the old mine workings and tailings still flows into Ullswater and Coniston Water.

Welsh and Denny (1980) investigated the uptake of Pb and Cu by nine taxa of submerged aquatic macrophytes from the two lakes. The macrophytic water-plant communities were dominated by rooted submerged species, which grow down to a maximum water depth of 5-6 m. The pH of the epilimnion varied from 6.0 up to about 8.0 in midsummer. Zones dominated by *Littorella uniflora* were common in the shallowest water, to be replaced by *Myriophyllum alterniflorum* and *Isoetes lacustris* at about 1-2 m depth. *Potamogeton* spp. tended to dominate between 3 and 4 m, and below this, charophyte species generally extended down to the greatest depth (5-6 m).

Collected plants were divided into root and shoot and rinsed three times in distilled water. Root and rhizome samples contained small indeterminate amounts of sediment particles which were not removed by the standard rinsing and agitation in distilled water. Similarly, epiphytic algal coverings on some shoots were only partially removed. DRIED sediments and plants were digested in concentrated $HNO_3$ (representing a partial total digestion for the sediment) and were analysed along with water samples for Cu and Pb concentrations.

Results showed that concentrations of Pb and Cu were high in the shoots (up to 720 µg/g dry weight for Pb, up to 510 µg/g for Cu) and roots and in the lake sediments (up to 40,000 µg/g of Pb, and up to 1,500 µg/g of Cu), but were low in the lake waters. Calculating Spearman rank correlation coefficients between variables, positive correlations were found between the metal concentrations in submerged shoots and in the underlying sediments, but no correlations were found between concentrations in shoots and in lake-water samples. Also, several significant relationships were obtained between root and shoot Pb and Cu concentrations (Table 1.11). It was concluded that the enrichment of metals in the shoots was largely derived from the high metal concentrations in the sediments. However, two different pathways for Pb and Cu were suggested by the authors for the transfer of metals from the sediments to the shoots.

**Table 1.11**. Correlation coefficients between the metal concentrations (µg/g) in shoots of submerged plant species, collected in Ullswater and Coniston Water lakes, and the metal concentrations in the roots and in the sediments (µg/g). From Welsh and Denny (1980).

| Shoot of *vs.* | Pb in | | Cu in | |
|---|---|---|---|---|
| | sediment | roots | sediment | roots |
| *Myriophyllum alterniflorum* | r= 0.76** | r= 0.59* | r= 0.44* | r= 0.56* |
| *Elodea canadensis* | r= 0.71** | r= 0.59* | r= 0.35 | r= 0.84* |
| *Potamogeton cripus* | r= 0.65** | r= 0.83** | r=-0.15 | r= 0.59* |
| *Potamogeton perfoliatus* | r= 0.48* | r= 0.44 | r= 0.06 | r= 0.13 |
| Characeae | r= 0.56* | - | r= 0.30 | - |

*P<0.05, **P<0.01

In earlier laboratory experiments on two submerged *Potamogeton* sp. (Welsh and Denny 1979), the authors found no evidence of any Pb translocation from roots to shoots, while Cu was clearly translocated in an acropetal direction. Both metals were rapidly accumulated from solution by shoot tissues. Thus, in the field study, the authors did not explain the relation between Pb in shoots and Pb in sediments by a possible direct Pb uptake by the roots followed by a translocation to the shoot, because the conclusion of the laboratory study did not validate this pathway. Lead accumulation in the shoots was considered to be the result of adsorption from the water. However, such a pathway of direct Pb uptake into the shoots from the lake water could not explain the correlation of Pb concentrations in shoots with that in the sediments, and the lack of correlation with Pb concentrations in the lake waters. The authors then postulated that the source of Pb for the shoots was some loss of Pb from the sediments to the immediately-overlying water, as a continuous immeasurable yet significant upward diffusion, or as periodic releases of Pb from the sediment effected by turbulence or lowered redox potentials.

Copper accumulation, on the other hand, was considered to be due mainly to absorption by the roots and translocation within the plant to the shoots. This conclusion is in accordance with the previous laboratory study, but not with the poor correlations obtained in the field between Cu concentrations in the shoots and in the sediments. Direct absorption from the water into the shoots was also postulated to occur. The observed similarity of the concentration of Cu in root and shoot tissues of plants supported this view, while for Pb, concentrations tended to be much higher in roots.

*Derwent Reservoir, England*

Harding and Whitton (1978) analyzed water, sediments and two species of submerged plants in Derwent Reservoir, Northern England. The most important source of metals (particularly Zn, Cd and Pb) in the reservoir came from an active fluorspar mine, via the drainage basin. The water level of the reservoir was subject to considerable fluctuations, according to rainfall, leading during dry periods to the exposure of large areas of contaminated mud colonized by *Gnaphalium uliginosum*, *Agrostis stolonifera*, *Juncus acutiflorus* and *J. effusus*. Macroscopic submerged plants were only occasional in the reservoir due perhaps, as postulated by the authors, in part to metal toxicity. The two most common submerged species encountered, and studied, were *Nitella flexilis*, a macroscopic alga, and *Glyceria fluitans*. Water, sediment and plant samples were analyzed for metals. The DRIED-sediment was digested for 30 min in boiling $HNO_3$. The plants were carefully washed in the reservoir water to remove attached debris, and washed again in the laboratory in double distilled water. *G. fluitans* samples were divided into leaves and stems; whole *N. flexilis* samples were analyzed.

Both species of plants showed marked accumulation of Zn, Cd and Pb and in general the variation in levels of these elements in the plants mirrored variations in water and sediments. Significant positive correlations were found between the Pb and Zn contents of *N. flexilis* and the «total» concentration of each metal in water at the time of sampling. Similar correlations were found for Pb of *G. fluitans* tissues, but no correlation was found between the Zn content of the leaves and that of the water. No significant correlations were found between the metal content of *N. flexilis*, which does not have true roots, and that of the sediment. *G. fluitans*, which has roots, showed a positive correlation between Pb in the sediments *vs*. Pb in the stems, while weaker correlations were found with the leaves. The authors concluded that *N. flexilis* probably accumulates almost all its metal content directly from the water, while the data obtained with *G. fluitans* suggest that sediments are a source of some of the metals accumulated in the plant tissues.

**Table 1.12**. Summary of the case studies section (section 1.3.1) concerning macrophytes and biomonitoring at mine sites.

| Location | Conclusions |
|---|---|
| Sudbury, Ontario | Reduction of the species diversity of macrophytes with decreasing distance from the smelters. *Leptodictyum riparium* (a moss) and *Eleocharis acicularis* were the most tolerant species to smelter pollution (Gorham and Gordon 1963).<br>High metal concentrations in lakes rather than acidity were responsible for the decrease in richness. *Eriocaulon septangulare* and *Eleocharis acicularis* were the most frequently encountered species in affected lakes (Yan *et al*. 1985).<br>Metal uptake was species specific (Hutchinson *et al*. 1975); Ni, Cu and Zn levels measured in *Potamogeton* tissues were considerably higher than those in the ambient water.<br>*Typha latifolia* inhabited all the contaminated wetlands visited by Taylor and Crowder (1983a). Ni and Cu concentrations were much higher in below-ground than in above-ground organs.<br>Zn and Cr concentrations were higher in *Eriocaulon septangulare* than in the other species studied (Reimer and Duthie 1993).<br>*Eriocaulon septangulare* was proposed by Miller *et al*. (1983) as a potential candidate for a biological indicator of metals; however, the |

| | |
|---|---|
| | authors were unable to link measured metal levels in plant tissues with total metal levels in the sediment. |
| The Moira River system, Ontario | Submerged rooted species accumulated much more metal in their tissues than floating and emergent species. Significant correlations were found between Ni, Co and Cu levels in *Myriophyllum verticillatum* and *Elodea canadensis* and the total metal concentrations in the sediment.<br>In the Bay of Quinte, Crowder *et al*. (1989) suggested the use of *M. spicatum* and *Vallisneria americana* as monitors of As contamination; however, *M. spicatum* accumulated much more Pb than *V. americana*. |
| Rouyn-Noranda, Québec | Campbell *et al*. (1985) studied the relationship between the partitioning of Cu and Zn in lake sediments and their availability to *Nuphar variegatum*. Compared with the total metal concentrations in the lake sediments, the levels found in the plant tissue were low. Zn accumulated in the plant tissues seemed to directly come from the water column, while the source of Cu seemed to be the sediment. The Cu fractions most readily extracted from the sediment gave better relationships with plant tissues than did total metal concentrations. Normalization of these readily extracted fractions with respect to the Fe content of the sediment further improved the relationships between Cu in sediment and in *N. variegatum* rhizome, suggesting that Fe may play a protective role in regulating Cu bioavalaibility; however, the usefulness of this ratio as a predictor of the sediment metal bioavailability for rooted macrophytes varied among metals and among plant species (Campbell and Tessier 1989). |

**Table 1.12** (continued)

| Location | Conclusions |
|---|---|
| The Northwest Miramichi and Tomogonops River system, New-Brunswick | Parts of the river affected by mining pollution showed a reduction in species diversity when compared to unpolluted parts. *Equisetum arvense* showed a wide ecological range, and did not appear to be as affected as other species by the mining operations (Besch and Roberts-Pichette 1970). |
| The Nepisiguit River system, New Brunswick | Ray and White (1976) concluded that *Oscillatoria* (an alga), *Potamogeton richardsonii* and *Equisetum fluviatilis* were useful as biological monitors to qualitatively reflect the long term water quality.<br>Cu and Zn concentrations in the tissues of *Equisetum arvense* qualitatively reflected the metal concentrations at the sampling sites; however, seasonal variations in the tissue metal concentrations existed and should be considered while using this species as a biological indicator (Ray and White 1979). |
| Flin Flon, Manitoba | *Myriophyllum exalbescens* was selected by Franzin and McFarlane (1980) as a potential indicator species, accumulating more metals in its tissue than the other species studied. However, they failed to find significant relationships between the metal content of the plant and the metal concentrations in lake waters and sediments, except for Zn in water, and |

**Table 1.12** (continued)

| Location | Conclusions |
|---|---|
| | suggested that the plant may qualitatively indicate the degree of Zn contamination of aquatic environments only if the contamination is severe. |
| Clark Fork River, Montana | Metal concentrations were much more elevated in roots than in leaves of *Typha latifolia*, and all contaminated sites showed elevated metal concentrations compared to the uncontaminated site. However, metal concentrations found in below-ground tissues may be associated with an external iron oxide coating known to adsorb metals (Johns 1987, 1993). |
| River Kokemäenjoki, Finland | *Nuphar lutea* was at first proposed by Aulio (1980) as a biological indicator of aquatic pollution; Cu concentrations in the plant parts were markedly elevated at sampling sites near the metal processing plant, and decreased sharply with increasing distance above and below the waste discharge point. The accumulation of Cu in various plant parts was clearly proportional to the Cu levels in the corresponding surface sediments. Later, Aulio (1986) concluded that the floating-leaved taxa appeared to have limited value as monitoring organisms, and favoured submerged *Potamogeton* species. |
| Lakes Ullswater and Coniston Water, England | Significant correlations were found between the metal concentrations in submerged shoots of several species and the metal concentrations in the underlying sediments, but no correlations were found with lake-water samples (Welsh and Denny 1980). The source of Pb for shoots of two submerged *Potamogeton* species was postulated to be some loss of Pb from the sediment to the immediately overlying water, Pb being taken-up directly from the water column. Cu accumulation by the plants was considered to be due mainly to adsorption by the roots from the sediments followed by a translocation within the plant to the shoots; direct absorption of Cu from the water into the shoot was also postulated to occur (Welsh and Denny 1979, 1980). |
| Derwent Reservoir, England | Harding and Whitton (1978) concluded that *Nitella flexilis* (a macroscopic alga without roots) probably accumulated almost all of its metal content directly from the water, while the data obtained with *Glyceria fluitans* (a rooted plant) suggested that sediments were a source of some of the metals accumulated in the plant tissues. |

### 1.3.2 Macrophytes in non-mine-related biomonitoring studies

Many researchers have studied the utility of using macrophyte species as biomonitor organisms of metal contamination in freshwater lakes as well as in estuaries.

The metal contamination did not come from mine activities but from different sources. Some of these papers will be reported briefly here, particularly the authors' conclusions about the usefulness of using macrophytes as biomonitor species.

Mayes and McIntosh (1975) exposed, in enclosures, shoots of *Ceratophyllum demersum*, a rootless, free-floating aquatic plant, collected from an uncontaminated control pond and transferred to contaminated ponds in northern Indiana, USA, and measured the accumulation of Cd and Pb in the plant tissues after 3, 6, 9 and 12 weeks of exposure. All experimental sites showed accumulation of Cd and Pb greater than in the control area. In a highly contaminated lake (Little Center Lake), maximum values of 20 µg/g Cd (initial concentration = 0.41 µg/g) and 260 µg/g Pb (initial concentration = 6.35 µg/g) were reached by *C. demersum*. The 12th week levels of Cd and Pb concentrations in the plant tissues were lower than the 6th or 9th week concentrations. A possible explanation given by the authors for this decrease in concentration at 9th or 12th week is that the growth rate was increasing more rapidly than the rate of metal accumulation, «diluting» the metals present and resulting in decreasing concentrations; a release of metals from the plants was also possible. Data showed a fairly large variation in Cd and Pb concentrations in plants among sampling stations within a same lake. Mayes and McIntosh (1975) concluded from their study that 1) *Ceratophyllum demersum* can be an effective monitor of contamination levels in lakes, 2) aquatic macrophytes may be of significant importance in controlling concentrations of biologically available metals due to their ability to remove metals from water (however, subsequent work would seem to indicate that this is unlikely in rivers (see St-Cyr *et al*. 1994) or in deep lakes, where phytoplankton play this role (see Sigg 1994)) and 3) the use of aquatic plants to remove contaminants from water may be influenced by the growth stage of the plants.

The possibility of transplanting hydrophytes for biological monitoring was also discussed by Ernst and Marquenie-Van der Werff (1978) in the Netherlands. The Cu content of plants collected in ditches near pig bioindustries (*Elodea nuttallii*, *Spirodela polyrhiza*, *Ceratophyllum demersum*, *Nuphar luteum*, *Vaucheria* sp. and *Oedogoneum* sp.)

was species-dependent and related also to exposure. Plant species with a well-developed rooting system such as *Nuphar luteum* had the highest Cu values in the roots, probably because of uptake from the mud. Three species were studied in laboratory and it was shown that Cu was incorporated in plant tissues by a multiphasic uptake, which depended on the concentration of the metal in the medium, temperature, pH, time, and the ratio of biomass- and water volume. Different species were differently affected by high metal concentrations and showed different resistance to the metal. The authors suggested that transplantation of *Elodea nuttallii* and *Callitriche platycarpa* as possible indicators of field Cu contamination could yield valuable information on pollution, especially in situations where water contamination occurs periodically. In contrast to chemical analysis of water collected at one particular moment in time, biological monitoring can integrate water quality over a period of time. Similarly, mud analyses, in view of the slow movement of metals in river sediments and ditch mud, cannot discriminate between recent and old pollution, but plants do (this statement by the authors is misleading, since by sediment coring, old and new sediments with their metal content can be distinguished by stratigraphy). The authors also reported that the free-floating species *Lemna minor* and *Spirodela polyrhiza* are not adequate for localizing water pollution.

Heisey and Damman (1982) investigated the effects of growth form (submersed, floating-leaved and emergent) on Cu and Pb uptake and determined the variation in Cu and Pb concentrations from plants collected in three rivers of eastern Connecticut, USA, subjected to different loadings of these two metals. The two most common species were *Pontederia cordata*, an emergent plant with well-developed rhizomes firmly rooted in the sediment, and *Potamogeton epihydrus*, a floating-leaved species with nearly its entire shoot biomass in contact with the water. Emergents with well-developed root systems in the soil normally obtain most of their mineral supply from the sediment, but, for those with free-floating stem roots, uptake from the water can also be important. Root and foliar uptake may occur for submersed and floating-leaved plants rooted in the sediment. Free-floating species not touching the bottom rely on the water (e.g. Delgado *et al*. 1993) and partly on the atmosphere. *Pontederia cordata* above- and below-ground parts were separately analysed, but the other species were handled as entire plants.

*Pontederia cordata* showed lower Cu concentrations than *Potamogeton epihydrus* and other floating-leaved and submersed macrophytes. Differences among growth forms were small for Pb. Concentrations of both elements were much higher in *Pontederia* under-ground (rhizomes and roots) organs than in shoots. Copper concentrations in *Pontederia* seemed to depend primarily on concentrations in the sediments, whereas Cu concentrations in *Potamogeton* and other floating-leaved and submersed species appeared more indicative of water quality, presumably due to foliar absorption. Lead concentrations in *Pontederia* seemed to be controlled primarily by uptake from sediments and atmospheric inputs. Differences in Pb concentrations of *Potamogeton* were small among rivers, indicating that foliar absorption and acropetal translocation of Pb from roots to shoot is low.

Heisey and Damman (1982) also collected the two dominant species at 3-week intervals, from July to November, to determine metal variation over time. Copper concentrations of *Pontederia* shoots dropped in late summer, coinciding with the period of most active growth, but returned to mid-summer levels in autumn. In *Potamogeton*, Cu concentrations changed very little over the season. Lead concentrations of *Pontederia* shoots declined slowly over the growing season, while no consistent trend was observed for *Potamogeton* from all sampling sites.

Brix *et al*. (1983) proposed the use of *Zostera marina* as a monitoring organism for metals in the Limfjord, Denmark. The concentrations of Pb, Cu, Cd and Zn were determined in above- and below-ground parts of this rosette-type form of plant collected at several locations in the shallow, brackish water of the Limfjord. The concentrations of Cd, Cu and Zn in above-ground parts were significantly higher than in below-ground parts; no significant difference was observed for Pb. Furthermore, a significant correlation between metal concentrations in above- and below-ground parts was found, indicating a relationship between metal availability in water and sediment respectively, or transport within the plant. It was suggested that the concentrations of some metals in above- and below-ground parts of *Zostera marina* could be used as a measure of the bioavailable fraction of these metals in ambient and interstitial water of the sediment respectively, while for other metals, translocation within the plant may be of some importance.

*Zostera marina* is abundant in these waters, widely distributed, tolerant to changes in salinity, able to accumulate metals, and the content of some metals in this plant reflects the levels in the sediment (Lyngby and Brix 1982). In the latter paper, it is reported that the concentrations of Pb, Zn and to a certain degree Cu in *Zostera marina* reflected the levels in the sediment, while the content of Cd seemed to reflect the Cd level in water. In another publication (Lyngby *et al*. 1982), the authors reported that the Zn content in above-ground parts of *Zostera marina* was mainly derived from the ambient water and the content in below-ground parts from the interstitial water of the sediment; translocation was extremely low.

Lyngby and Brix (1982) also studied the seasonal variation of these metals in *Zostera marina* and observed a general seasonal variation pattern, the same for the two essential (Cu, Zn) and the two non-essential (Cd, Pb) metals studied, in above- and below-ground parts of the plant. Maximum concentrations were encountered in late winter - early spring and minimum concentrations in the autumn. This pattern was associated with the growth dynamics of the species: maximum concentrations were recorded when the growth had ceased, while distinct declines were observed at the beginning of the growth season. The authors suggested that the decrease in metal concentrations was probably due to the increase in biomass (growth dilution).

Mortimer (1985) reported a five year study in the Ottawa River, where the ability of freshwater aquatic vascular plants to accumulate metals, and their use as biological monitors, was examined. Field studies were done in combination with laboratory studies. Mortimer (1985) suggested that the observed changes in accumulation rates of metals as a function of the season and the physiological age of the plant, the localization of the accumulated metal in growing parts, and the effect of metal concentration on the uptake rate need to be studied in the laboratory before aquatic plants can be used to monitor metal concentrations in water. Laboratory studies to establish uptake rate constants of Hg (inorganic and methylmercury) by *Elodea* allowed the use of plants sampled in the field for estimating the amount and kind of Hg in the water. In the study area, background levels of Hg in aquatic plants of 35-50 ng/g dry weight corresponded to a water concentration near 15 ng/L of total mercury, of which 25-30% was methylmercury. Higher concentrations of Hg in the plants indicated a proportional increase in the Hg level in the water. From earlier laboratory experiments (Eriksson and Mortimer 1975), it was concluded that Hg was absorbed from the water into the submerged green parts of the plant, and subsequently transported to the roots within the plant.

Copper, Ni and Pb were also studied by Mortimer (1985), in a variety of aquatic plants collected in the field and studied in the laboratory. The author concluded that the higher atomic weight ions (as Pb and Hg) were accumulated much more effectively by the plants than lower atomic weight elements, such as Ni and Cu. Differences in uptake rate were found to depend on the plant species, the seasonal growth rate changes and the metal ion being absorbed. Each of the metals had its own characteristics and each plant species accumulated differently. Mortimer (1985) concluded that «the relation between the heavy metal content of rooted aquatic plants and the sediment in which they are growing is by no means direct. The accumulated evidence from this study indicates that for heavy metals there is probably no relation based on root absorption and upward transport. The experimental evidence suggests that movement in the reverse direction is more dominant. That is, metals contained in the water can be absorbed by the submerged stems and leaves, transported to the roots and added to the sediment concentration when the roots die.» This general conclusion given by Mortimer (1985), who worked mostly with Hg uptake by aquatic plants, is rather surprising in that it is in contradiction with most other researchers, who consider that root uptake of metals from the sediment followed by upward transport to the shoot effectively occurs for several metals (Cu: Welsh and Denny 1979, Campbell *et al*. 1985; Cd: Mayes *et al*. 1977, Brinkhuis *et al*. 1980; see also St-Cyr and Campbell 1997 (p. 56) and Greger and Kautsky 1993, next paragraph, who reported that metal transport from shoots to roots is low).

Greger and Kautsky (1993), in the waters near Stockholm, Sweden, suggested that since several factors affect the bioavailability of metals in the aquatic environment (e.g. pH, organic matter, salinity, redox potential), it is impossible to measure the bioavailability by sediment analysis. However, all these parameters will be integrated when macrophytes take up metals, and since macrophytes take up metals from the sediments by the roots and directly from the water by the shoots, the integrated amounts of bioavailable metals in water and sediment can be indicated by using macrophytes. The metal content in shoots is the sum of the net amount taken up from the water and the net amount transported from the roots. Metal contents in roots depend mainly on the amount taken up from the sediment, because transport from shoots to roots is low. Thus, using macrophytes along with analyses of sediment should be a valuable tool when mapping the bioavailable amounts of metals in shallow coastal areas as well as in lakes.

*Potamogeton perfoliatus*, being the most frequently occurring species in the studied area, was proposed by Greger and Kautsky as the most useful species for their study; to compare data between localities, the same species must be used. However, *Myriophyllum spicatum* accumulated much higher amounts of Zn, Pb and Cd in its tissues than the other submerged plant studied (*P. perfoliatus* and *P. pectinatus*). The authors reported that different plant species take up metals in different amounts, and may also transport metals from the roots up to the shoots to various degrees; thus they suggested the use of whole plants (above- + below-ground parts) of the same species to be able to compare data from different places.

St-Cyr and Campbell (1994) suggested the use of *Vallisneria americana*, a rosette-type submerged plant, as a potential biomonitor species reflecting metal contamination in the St. Lawrence River. This plant grows in most submerged plant communities and in all water types in the St. Lawrence River. *Vallisneria americana* concentrated metals (other than Pb; *cf*. Crowder *et al*. 1989, section 1.3.1.2) in its above-ground tissues to higher levels than did *Potamogeton richardsonii*, another abundant submerged rooted species in the studied area. It should be noted that despite this difference in metal bioconcentration in the above-ground parts of these two plants, the two species followed the same spatial trends, showing elevated metal concentrations at the same stations. Both species reacted similarly to changes in metal levels in their environment in the study area. The metal content of the leaves of *V. americana* reflected spatial variations in environmental contamination in the study area and represented the bioavailable metals potentially transferable through the food chain to higher organisms (St-Cyr and Campbell 1994). However, for biomonitor species, an important criterion to be met is also to show a predictable and mechanistically understandable relation between the accumulated metal content in their tissues and the levels of «bioavailable» metals in the surrounding environment; these relations should be robust *i.e.* consistent over time (interannual variation) and space (in different study areas) (Phillips 1977).

These relations were investigated by St-Cyr and Campbell (1997). *Vallisneria americana* was collected in four fluvial lakes of the St. Lawrence River system (Lake St-François, St-Louis, St-Pierre and des Deux Montagnes) over two years. Collected plants were separated into roots and above-ground parts, exclusively composed of ribbon-like leaves. Roots were treated with DCB (sodium dithionite - sodium citrate - sodium bicarbonate) before metal analysis to remove external contamination; leaves were carefully washed with deionized water. Samples of the superficial layer (about the first cm) of bottom sediment, taken near collected plants, were subjected to the sequential extraction procedure of Tessier *et al*. (1979), as described in Campbell *et al*. (1985) in the Rouyn-Noranda case study, to determine the partitioning of metals at the sediment/water interface. As far as possible, the dissolved free-ion concentrations of metals in the sediment interstitial water were estimated. Previously published equations to estimate [$Cd^{2+}$] (Tessier *et al*. 1993) and [$Pb^{2+}$], [$Cu^{2+}$], [$Ni^{2+}$] and [$Zn^{2+}$] (Tessier 1992) were used, using as parameters concentrations of potentially available sediment-bound metal, concentrations of Fe oxyhydroxides, both obtained from chemical extractions, and the sediment pH. For the [$Cd^{2+}$] determination, sediment organic carbon concentrations were also taken into account.

Sediment seems to be an important source of metals for the plant. However, total metal concentrations in the sediment are not a useful measure to predict metal uptake by the plant; the free-ion concentration estimates are much more realistic. For example, although no significant relation was obtained relating Cd concentrations in roots of *Vallisneria americana* with the total Cd concentrations in the underlying sediment (r = -

0.108, P>0.05), this relation improved if the estimated free Cd concentrations ([$Cd^{2+}$]) at the sediment/water interface was considered (r=0.541, P<0.01). Similar results were observed for Pb, where correlations with total Pb in sediment gave r=0.026, P>0.05, while with the estimated [$Pb^{2+}$] the correlation was much greater (r=0.713, P<0.01; Fig. 2).

**Figure 2**. Relationships between Pb concentrations (µg/g ± standard-deviation) in roots of *Vallisneria americana* collected in 1990 in the St. Lawrence River *vs*. total Pb concentrations in the sediment (µg/g) and the estimated free Pb concentrations ([$Pb^{2+}$]) at the sediment-water interface (mol/L). From St-Cyr and Campbell (1997).

However, no significant relations were obtained relating [Zn], [Cu] and [Ni] in *Vallisneria* tissues with respectively [$Zn^{2+}$], [$Cu^{2+}$] and [$Ni^{2+}$] in the sediment interstitial water (there is no published equation to estimate [$Cr^{3+}$]). These equations need to be refined to better reflect the free-metal concentrations of the interstitial water. Other variables than those already mentioned (partitioning of metals in sediments, Fe oxyhydroxides) may also contribute to explain metal uptake by aquatic plants, such as dissolved Ca concentrations and sediment organic carbon content (St-Cyr and Campbell 1997).

**Table 1.13**. Correlation coefficients between the metal concentrations (µg/g) in roots of *Vallisne- ria americana* collected in the St. Lawrence River and the metal concentrations in the sediment, as total concentrations (µg/g) and the ratio [M(S3)]/[FeF3]. From St-Cyr and Campbell (1997).

| *Vallisneria americana* | | sediment | |
|---|---|---|---|
| roots (µg/g) | N | Total (µg/g) | [M(S3)]/[FeF3]* |
| Zn | 12 | r = 0.608, P<0.05 | r = 0.884, P<0.001 |
| Cu | 30 | r = 0.002, P>0.05 | r = 0.396, P<0.05 |

*This ratio [M(S3)]/[FeF3] corresponds to the sum of metal concentrations (µg/g) in the three first fractions extracted from the sediment, following the Tessier *et al*. (1979) procedure, divided by the Fe concentration (µg/g) in sediment Fraction 3.

In the case of Zn and Cu, normalizing the bioavailable metal concentrations in the sediment in respect to the Fe oxyhydroxide concentrations improved the relation between these metals in *Vallisneria* roots and in sediment (Table 1.13). This role of sediment Fe oxyhydroxides controlling metal availability to various aquatic species had been already reported (Campbell *et al*. 1985; Tessier and Campbell 1990; Campbell and Tessier 1996). A similar relation was also obtained when relating Zn concentrations in the root iron plaque of *Vallisneria* with internal Zn root concentrations (St-Cyr and Campbell 1996). There is some geochemical basis for this improved relation, in that the [Zn]/[Fe] or [Cu]/[Fe] ratio can under certain circumstances be considered as a surrogate measure for the free [$Zn^{2+}$] and [$Cu^{2+}$] in the interstitial water surrounding the root (Campbell and Tessier 1989; 1996).

For Ni and Cr, the results obtained in the St. Lawrence River system indicate that the most readily extractable forms of Ni and Cr in the sediment ([NiS3] and [CrS3], that is the sum of Ni and Cr concentrations in the three first fractions extracted from the sediment) can be used as predictors of these metal concentrations in *Vallisneria* roots (Table 1.14).

**Table 1.14**. Correlation coefficients between the metal concentrations (µg/g) in roots of *Vallisneria americana* collected in 1990 in the St. Lawrence River and the metal concentrations in the sediment, as total concentrations (µg/g) and the sum of metal concentrations in the three first fractions extracted from the sediment [M(S3)] (µg/g). From St-Cyr and Campbell (1997).

| *Vallisneria americana* | | sediment | |
|---|---|---|---|
| roots (µg/g) | N | Total (µg/g) | [M(S3)] (µg/g) |
| Ni | 17 | r = 0.454, P>0.05 | r = 0.548, P<0.05 |
| Cr | 17 | r = 0.526, P<0.05 | r = 0.531, P<0.05 |

Cadmium and Pb concentrations were generally higher in the root tissues than in the leaves; the same is true for Cu and Cr, but with more variability. Zinc and Ni concentrations however, were higher in the leaves than in the roots (St-Cyr and Campbell 1997). Positive relationships were found between [Cd], [Pb] and [Zn] in leaves, with these same metals in the roots, and with the estimated bioavailable fraction in the interstitial water (Table 1.15). For Ni and Cr, more pronounced in the case of Cr, no significant relationships were found between metals in leaves and in roots. Chromium appears to be rather immobile.

**Table 1.15**. Correlation coefficients between the metal concentrations (µg/g) in leaves of *V. americana* collected in the St. Lawrence River and the metal concentrations (µg/g) in roots and the best relationship obtained with some measures of bioavailable metal concentrations in the sediment (N=12 to 30, depending of the metal). From St-Cyr and Campbell (1997).

| *Vallisneria americana* | | Bioavailable sediment fractions | |
|---|---|---|---|
| Leaves (µg/g) | Roots (µg/g) | | |
| [Cd] | r = 0.614, P<0.001 | r = 0.598, P<0.001 | [$Cd^{2+}$] (mol/L) |
| [Pb] | r = 0.722, P<0.01 | r = 0.537, P<0.05 | [$Pb^{2+}$] (mol/L) |
| [Zn] | r = 0.697, P<0.05 | r = 0.609, P<0.05 | [Zn(S3)]/[FeF3] |

| | | | |
|---|---|---|---|
| [Cu] | r = 0.509, $P<0.01$ | not significant | |
| [Ni] | r = 0.487, $P>0.05$ | r = 0.625, $P<0.01$ | [Ni(S3)] (µg/g) |
| [Cr] | r = 0.117, $P>0.05$ | not significant | |

Over the study area, one of the sampling stations was localized near the outlet of the Richelieu River, a known point-source of contamination in the water column, receiving the effluent discharges of the Sorel-Tracy industrial complex. It seems that metal concentrations (Cr, Cu, Ni, Pb and Zn) in the water column were higher there than at the other stations sampled. Metal contributions from the surrounding water *via* leaf uptake seem to be important at this station, as graphically illustrated in St-Cyr and Campbell (1997). Over the study area, Cu uptake from the surrounding water *via* the leaves seems to be important, particularly comparing stations localized in different water masses (in Lake St-Louis, in waters coming from Lake St-François and those from Lake des Deux Montagnes). This foliar contamination is suspected to be combined with a probable basipetal translocation of Cu, since there is a good relation between Cu concentrations in leaf and root tissues, which contributed to a high variability in the root Cu data, and attenuated potential relationships between [Cu] in roots and [Cu] in the sediment. No significant relationship was found between leaf Cu concentrations and any of the sediment Cu fractions analyzed.

It was concluded from these results that:

i) *Vallisneria americana* offers good potential as a biomonitor species, reflecting metal contamination in sediment and in the water column;

ii) Roots are better bioindicator organs than shoots to reflect sediment contamination. For above-ground parts of the plant to be used as monitors of sediment metals, a translocation of metals from roots to shoots must happen (excluding immobile elements such as Cr) and no foliar contamination must occur. Foliar contamination from, for example, point-source pollution of the water obscures the relationships with sediment metal concentrations, and if basipetal translocation occurs, increases the variability in root metal concentrations. However, in the context of a biomonitoring species chosen to integrate the overall impact of a mine effluent, plant roots AND foliage uptake of metals will then represent an advantage.

## 1.4 CRITICAL EVALUATION OF THESE AND OTHER POTENTIAL MONITORING METHODS

### 1.4.1 Species composition of plant communities

When performing a survey of contaminated waters due to mine activities, one can first establish a list of macrophyte species presence/absence. The absence of submerged/emergent plants from streams/water bodies which would normally support them (when compared to control areas not affected by mine activities) can be regarded as an indication of general pollution due to mining activities (Gorham and Gordon 1963; Besch and Roberts-Pichette 1970; Yan *et al*. 1985).

Lists of plant species present along a contamination gradient due to a heavy polluted source, as near a smelter, have often been prepared, as for other environmental stresses, and some common features emerge (Kelly 1988; Sudbury case study):

1) the decline in species number;

2) increases in the density of the few tolerant species able to thrive with the reduced competition;

3) gradual changes in community composition along environmental gradients as the pollution decreased.

Downstream recovery of the macrophyte flora was also observed below lignite mines in Denmark (Sand-Jensen and Rasmussen 1978). At the most upstream sites, with a pH between 3.0 and 4.0, the flora was generally restricted to bryophytes (*Anisothecium vaginale*, *Polytrichum longisetum*) and emergent angiosperms (*Phragmites australis, Juncus bulbosus*, *J. effusus*); however, further downstream, as pH and alkalinity rose, submerged angiosperms such as *Elodea canadensis* and *Callitriche cophocarpa* were able to grow (Kelly 1988). Acidity is associated with acid mine drainage due to mining operations. However, it seems that it is the metal content of metal-contaminated acidic lakes, rather than the acidity in itself, which has a negative effect on macrophytes (Yan *et* *al*. 1985). The liming experiment of Jackson *et al*. (1990) in Bowland Lake in the Sudbury area revealed that the changes in pH and alkalinity following neutralization (pH 4.8-5.5 increased to 6.3-6.7) did not substantially affect the macrophyte community of the lake. However, increased percent shoreline coverage was noted for most species two years following liming, and two uncommon species observed prior to neutralization (*Eleocharis parvula* and *E. acicularis*) were not observed afterwards, while two new and uncommon species (*Nitella* sp. and *Brasenia schreberi*) were (Jackson *et al*. 1990).

Small *et al*. (1996) proposed a macrophyte-based biosurvey to estimate stream water quality. The purpose of the study was to expand the European method of using macrophytes as indicators of stream water quality, principally in regard to eutrophication, a largely qualitative method emphasizing indicator species (Haslam 1982; Stanley 1986) to a method permitting statistically significant separation of streams in accordance with their water quality rating. The method for monitoring river pollution developed by Haslam (1982), now largely used in England, considers important criteria such as species diversity, vegetation cover, trophic status, pollution tolerance and physical damage. These are assessed against the vegetation expected in a similar clean stream under traditional management. Basically, the Small *et al*. (1996) method also consists of comparing the higher plant communities associated with streams that are known to be of high quality with those of known degraded systems. Unlike organisms extensively used in biomonitoring techniques (e.g. aquatic insects and their larvae), macrophytes are considerably easier to identify and to quantify. In addition, the macrophyte technique provides a range of measures of increasing sensitivity from rough counts of species numbers at a few sites, to the presence/absence and abundance of certain indicator species, and finally to a diversity analysis based on easily identified species at an extended number of sites. The study was done in the Chesapeake Bay watershed, which is experiencing problems of eutrophication and non-point source chemical pollution.

Species found at known polluted and non-polluted sites were first listed. Rarely seen species or those difficult to identify were eliminated. Indicator species of high and low water quality were determined; to do so, macrophyte community structure was examined at the species level by looking at the abundance and frequency of occurrence of each species in high and low- quality streams. Four diversity indices were then calculated and combined, given measures of richness, evenness and abundance. Small *et al*. (1996) proposed to apply these methods to all streams in a large watershed, and then more subtle analyses could be done to verify and identify specific pollution sources in a more limited number of problem streams. Tributaries showing moderate to low water quality can be cross-checked with other monitoring methods (e.g. invertebrates or diatom biomonitoring). Finally, biomarker or bioassay techniques can be employed in specific cases in which chemical toxicity is suspect.

*Such a procedure could perhaps be applied at mine sites to determine the area affected by mine activities and to follow over time the restoration of the sites.*

Sortkjaer (1984) reported a German paper (Kutscher and Kohler 1976) where a simple monitoring system was described based on four zones according to the degree of pollution. Each zone is characterized by one species of macrophyte or a group of such species, as follows:

Zone A: *Potamogeton coloratus*

Zone B: *Sium erectus*

Zone C: *Potamogeton densus*

Zone D: *Callitriche obtusangula* and *Ranunculus fluitans*

The classification of Kutscher and Kohler (1976) was based on observations made on a single river system, but later they extended it to three other rivers and found it to be useful there, although Zones C and D were better combined.

Clements (1991) reported that our understanding of how metals affect natural populations, communities, and ecosystems in the field is greatly limited. Field investigations on the impact of metals generally fall into two categories: descriptive and experimental. Descriptive approaches, such as routine biomonitoring of community structure and measurement of levels of metals in organisms, are the most commonly employed techniques for assessing the biological integrity of streams. Biomonitoring approaches are often limited by the lack of appropriate spatial and/or temporal controls, therefore making it difficult to attribute observed changes to specific causes. Stream biomonitoring approaches for evaluating the impact of contaminants typically involve comparison of upstream reference sites to downstream impacted and recovery sites. Ideally, these reference locations should be similar in all respects except for the presence of contaminants, but this condition is often difficult to satisfy.

As already reported in the case studies (section 1.3), environments near mine activities where plants live are often harsh, with such characteristics as rough substrates, little organic matter deposits, irregular water levels, acidity, poor nutrient (N, P) levels, not to forget high metal concentrations and often high turbidity and suspended solids. There have been many studies on the effects of metals and acidity on the aquatic ecosystem, not considering all these associated effects, which can have severe consequences on inhabitants of the affected water bodies. Plants living in these conditions must be able to tolerate not only high metal concentrations, but also other adverse conditions. With increasing distance from the point-source disturbance, these difficult conditions are normally progressively attenuated. Even if a plant can overcome the acidity itself and the iron oxide precipitate often encountered in acid mine drainage, it still has to be able to grow in the absence of bicarbonate. It is not surprising then to find that the two most abundant groups of macrophytes in non-treated acid-mine drainage streams are the bryophytes and submerged plants which are able to use carbon dioxide rather than bicarbonate as their inorganic carbon source, and emergent angiosperms, which are not wholly dependent upon the inorganic carbon supply in the water (Kelly 1988).

Some of the most frequently cited macrophytes to be encountered in environments perturbed by mine and smelting activities (excluding mosses; see the case studies and Ehrle 1960; Kelly 1988) are:

| EMERGENT | SUBMERGED |
|---|---|
| *Typha latifolia* | *Eriocaulon septangulare* |
| *Phragmites australis* | *Isoetes muricata* |
| *Eleocharis acicularis (can be submerged)* | *Lobelia dortmanna* |
| *Eleocharis smallii* | *Potamogeton richardsonnii* |

| | |
|---|---|
| *Juncus pelocarpus (can be submerged)* | |
| *Juncus effusus* | FLOATING-LEAVED |
| *Glyceria borealis* | *Nuphar variegatum* |
| *Equisetum arvense* | *Nymphaea odorata* |
| *Equisetum fluviatile* | |

Bryophytes are often conspicuous in acid streams, affected by acid mine drainage (Gorham and Gordon 1963; Sand-Jensen and Rasmussen 1978) and have been suggested for monitoring metal pollution (Say *et al*. 1981; Mouvet 1985).

One of the predominant changes experienced by water bodies near mine activities, and affected by acid mine drainage, is acidification of the water, which affects the physiology of macrophytes, particularly with changes in the availability of carbon, a change from nitrate to ammonium as a nitrogen source and the effects of an alteration in the lake light climate. Also, in acid water, some metal ions in the water column are more available to organisms (Campbell and Stokes 1985) and may be selectively accumulated in the species that are dependent on the water column for nutrient uptake. Lakes with acid water (e.g. pH 4.6-6.5) are characterised by a dominance of the isoetids (*Lobelia dortmanna*, *Littorella uniflora*, *Isoetes* spp.), *Juncus*, *Nymphaea*, etc... (Farmer 1990). *Eriocaulon septangulare* is reported to frequently co-occur with *Lobelia dortmanna* (Marie-Victorin 1964). At much lower pHs, the isoetids no longer are present, but lakes can become dominated by *Sphagnum* (Farmer 1990). The successful growth of the isoetid species under the extremely low concentrations of inorganic carbon in the lake water can be ascribed to their ability to take up inorganic carbon from the sediments, via their roots (Søndergaard and Sand-Jensen 1979). Some other species are plastic in their ability to use $HCO_3^-$ or $CO_2$ dependent of the environment in which they grow (e.g. *Elodea canadensis*; Sand-Jensen and Gordon 1986).

### 1.4.2 Metal accumulation in plant tissues

The determination of metal concentrations in plant tissues is widely used, as seen in the case studies (section 1.3), as a method to monitor and assess metal distributions and availability in contaminated environments. Important characteristics for biological indicators that are to be employed to monitor metals include (from Phillips 1977, 1980; Hellawell 1986):

- the organism should be readily identified (taxonomic uncertainties can confuse data interpretation);
- the organism should be sampled easily (without the need for several operators or expensive equipment), and quantitatively;
- the organism should have a cosmopolitan distribution (for example, the absence of species with very narrow ecological requirements and limited distributions may not be associated with pollution);
- the organism should be sedentary in order to be representative of the study area;
- the organism should be abundant throughout the study area;
- the organism should be of reasonable size, giving adequate tissue for analysis;
- the organism should be associated with abundant autecological data. The basic biology/physiology of the biomonitor organism should be known so that sources of uncontrolled variation (seasonal growth, reproduction) can be minimized;
- the organism should readily accumulate metals, without being adversely affected by the levels encountered in the environment;
- the organism should be sufficiently long-lived, so as to integrate environmental exposures of at least several months;
- the organism should exhibit a high concentration factor for metals, allowing direct analysis without pre-concentration;
- the organism should be easily cultured in the laboratory, allowing controlled studies of metal uptake;
- a simple relation should exist between the metal content of the organism and the average metal concentration in the surrounding environment;
- all organisms of a given species used in a survey should exhibit the same correlation between their metal contents and those in the surrounding environment at all locations studied, under all conditions.

Benefits of employing tissue analysis include (Phillips 1980):

- concentrations of metals are often greater in tissues than in water because of bioconcentration and are thus easier to analyse, and also increases the probability of detecting trace amounts of metals in the environment;
- measurements of metal concentrations in tissues provide a time-averaged assessment of the metals.
- concentrations of metals in tissues provide direct measurement of bioavailability of those metals that accumulate in the tissues; it is a direct determination of metal bioavailability, which is not necessarily a simple function of the total metal concentrations in water and sediment.

Macrophyte species possess several of the characteristics of the «ideal» biomonitor. Macrophytes are stationary (in contrast to fish, for example) and visible to the naked eye (in contrast to phytoplankton or zooplankton, for example), and they are easy to collect and to handle (e.g. easier to collect than burrowing insect larvae); some species are widespread and some of the more common species have been studied in tissue biomonitoring of metal contamination. The demands on manpower for macrophyte surveys are low. This is a group of organisms showing considerable potential for environmental impact assessments. However, in temperate climates, they are subject to marked seasonal variations in biomass and are available during a relatively short growing period (Hellawell 1986).

Whitton *et al*. (1981) noted in favor of plants that:

- plants have the ability to concentrate metals in their biomass at levels of three to four orders of magnitude greater than levels in the ambient environment, thereby increasing the sensitivity over analysis of water;
- plants have the ability to give a time-integrated picture of contaminant concentrations;
- plants reflect the bioavailable fraction;
- plants are easy to store and preserve;

- data from plants may be easier to interpret than data from animals which may be complicated by differing diets and avoidance behaviour.

Whitton *et al*. (1981) suggested the use of ten macrophyte species (including the macro-algae *Cladophora glomerata*, *Nitella flexilis*, *Enteromorpha* and *Lemaneae fluviatilis*, the mosses *Amblystegium riparium* (syn. *Leptodictyum riparium*), *Fontinalis antipyretica*, *Fontinalis squamosa* and *Rhynchostegium riparioides*, the liverwort *Scapania undulata* and the angiosperm *Potamogeton pectinatus*) to monitor the levels of metals in European river and stream waters. Restricting the number of species chosen renders it possible to accumulate considerable background information about each of them, and to compare results obtained in one area with another. According to Whitton *et al*. (1981), these plant species should be sufficient to cover the majority of sites, while at the same time being few enough for everyone concerned to become thoroughly familiar with the organisms. The authors suggested that the use of these ten plants be regarded as standard practice (Whitton *et al*. 1981).

Even if up to now few statutory pollution-control authorities have included macrophyte species in their routine monitoring procedures, in the guidelines for studies of contaminants in biological tissues of the National Water-Quality Assessment (NAWQA) program of the U.S. Geological Survey, macrophytes were evaluated to determine their suitability as target oragnisms for NAWQA tissue analysis sampling, along with mollusks, fish, aquatic insects and crayfish. Three genera of rooted submerged macrophytes were retained in their routine procedure (leaves and stems from the apical 5 cm of *Potamogeton* sp., *Hydrilla verticillata* and *Elodea* sp.; Crawford and Luoma 1993).

Among the plant species selected by Whitton *et al*. (1981) and Crawford and Luoma (1993) as potential biomonitor organisms, some have been reported to occur in Canadian environments affected by mining and smelting activities (Sudbury, Ontario (section 1.3.1.1): *Leptodictyum riparium*, *Potamogeton epihydrus* v. *nuttallii*, *Potamogeton* sp., *Fontinalis* spp.; Rouyn-Noranda, Québec (section 1.3.1.3):

*Potamogeton richardsonii*; The Nepisiguit River system, New Brunswick (section 1.3.1.5): *Potamogeton richardsonii*).

*In the present state of knowledge about the most frequently reported species in the Canadian case studies (section 1.3.1), three frequently encountered and undoubtedly interesting species to look for as biomonitor organisms in the Canadian environment would be: Eriocaulon septangulare, Eleocharis acicularis and Potamogeton richardsonii.*

1.4.2.1 Plant parts to be analysed and cleaning methods

The choice of which part of the plant to analyze for metal determination, for eventual correlation with environmental contamination, is important.

<u>Below-ground parts: roots and rhizomes</u>

The below-ground parts of the plant must be analysed when the availability of metals in sediments is to be evaluated (Lyngby and Brix 1982; Campbell *et al*. 1985; Campbell and Tessier 1989). Some researchers (e.g. Campbell *et al*. 1985) have chosen to analyze rhizomes to do so. However, as already mentioned in the case studies section (Rouyn-Noranda), because of redistribution along the phloem from the rhizomes in an upward direction in the spring, and downward direction from the green parts to the rhizomes, in late summer, of carbon reserves, nutrients and possibly metals, rhizome analysis does not give an instant snap-shot view of what is taken up from the sediment by the plant. Analysis of root tissues would better reflect what is actually taken up by the plant.

Analyzing submerged plants for metals, researchers often have neglected the roots, and thought, as suggested by Eriksson and Mortimer (1975), that: «It appears that in surveys concerned with total enrichment of heavy metals in aquatic plants, the exclusion of underground parts may not be an important mistake in submersed species, as they often have a very small biomass proportion in their underground parts.» So, either the roots are removed and not analysed at all (e.g. Eriksson and Mortimer 1975; Franzin and McFarlane 1980) or analysed together with the green parts of the plant (Mudroch and Capobianco 1979; Greiger and Kautsky 1993; Reimer and Duthie 1993, to cite only those studies already listed in the case studies section). However, above- and below-ground parts do not accumulate metals to the same extent (Brix *et al*. 1983; St-Cyr and Campbell 1997), and probably not from the same source. Metals in roots should reflect the metal availability of the sediment. If foliar contamination directly from the water column occurs, and if basipetal translocation of the metal occurs, then metals taken up by the shoot will contribute to the metal content of the roots; this leads to an increased variability in metal concentrations in the roots, which can obscure the detection of relationships between metal in roots and in sediment (St-Cyr and Campbell 1997).

However, it is true that roots often represent a very small biomass, particularly in submerged plants, and that these roots are often contaminated with iron deposits/sediment particles (Welsh and Denny 1980; Taylor and Crowder 1983a; Johns 1987, 1993; St-Cyr and Campbell 1996). The occurrence of iron oxyhydroxides as coatings on roots has been widely observed in aquatic plants (Crowder and St-Cyr 1991). Two main factors are thought to explain the accumulation of iron oxyhydroxides on these roots: (i) the greater availability of soluble ferrous iron species in submerged soils and (ii) the radial loss of oxygen from the roots. To circumvent the problem of having roots growing under reducing conditions, aquatic plants have developed a ventilation system or aerenchyma that acts as a conduit for the transport of $O_2$ from the shoot to the root tissues, where it supports root respiration; some of this $O_2$ leaks outside the root, to the rhizosphere. When ferrous iron of the sediment interstitial water encounters $O_2$ in the rhizosphere, it is subject to oxidation and precipitation as ferric oxyhydroxide on the root surface (Armstrong 1979, 1982). In emergent and floating-leaved plants, the source of $O_2$ for the roots is the atmosphere whereas in submerged species, the source of $O_2$ is photosynthesis (Sand-Jensen *et al*. 1982; Thursby 1984). In turn, these iron oxyhydroxides can bind other nutrients and metals. If not properly removed, these iron coatings can swamp the true metal content of the roots (St-Cyr and Campbell 1996).

In aquatic plants, roots are frequently reported to contain higher concentrations of most metals than above-ground parts (Outridge and Noller 1991). However, elevated metal concentrations in roots of aquatic plants, as reported in the literature, will tend to reflect both internal concentrations of metallic ions and external contamination, including metals associated with the plaque. These high amounts of metals outside the root tissues can potentially confound relationships between root metal content and sediment metal content, if not properly removed. This is particularly true if these relations are sought at the end of the growing season, since the iron plaque accumulates on the roots throughout the summer (Crowder and Macfie 1986). The effectiveness of various techniques for the extraction of iron plaque from plant roots was tested by Taylor and Crowder (1983c) and McLaughlin *et al*. (1985). The DCB (sodium dithionite - sodium citrate - sodium bicarbonate) method, originally designed for the extraction of iron oxides from soil material, has proved to be efficient to remove the iron oxyhydroxide coating without damaging root tissues (Otte *et al*. 1989; St-Cyr and Campbell 1996). Other methods, such as rinsing roots with deionized water or washing with synthetic chelates EDTA and DTPA, were all inefficient, leaving considerable Fe on the surface of the washed roots.

Above-ground parts: stems, leaves and reproductive organs

Contamination, as for the roots, is also reported to occur on the leaves of submerged aquatic plants (e.g. Welsh and Denny 1980; St-Cyr and Campbell 1997), due to the presence of periphyton or aufwuchs (Newman and McIntosh 1989), which in itself could be used as a monitor of metal contamination (see the section 3 of the report on periphyton), and also to the precipitation of calcium carbonate on the leaves (Borowitzka 1984) which can potentially adsorb metals (Kelly and Ehlmann 1980). Various methods reported in the literature, which consist of immersing green plant parts in mild acidic solutions (dilute hydrochloric acid) for variable times, lead to damage of the leaf tissues (Azcue *et al*. 1993; St-Cyr and Campbell 1997). A clean-up procedure for the leaves, as developed for the roots, to completely remove carbonate incrustations and other impurities on the surfaces, is not yet available because of the delicate nature of these tissues. The method currently recommended is to carefully wash the leaves with deionized water, knowing that this procedure does not clean the leaves entirely, and the more the leaves are dissected (e.g. in *Myriophyllum* sp.), the more difficult it is to clean them.

In submerged rosette-type plants (e.g. *Eriocaulon septangulare*, *Vallisneria americana*), the above-ground parts consist only of leaves (if we exclude flowers and fruits); the stem is tiny. However, in other submerged species such as *Potamogeton richardsonii* and *Myriophyllum spicatum*, the above-ground parts of the plant are composed of a long branched stem bearing many small leaves. In these plants, metal concentrations in the leaves can be up to two or three times higher than in the stem (Louise St-Cyr, unpublished results for *Potamogeton richardsonii*; Kovacs *et al*. 1984 for *P. perfoliatus*). However, in mature plants, stem biomass is higher than leaf biomass. Thus, plants having a long stem tend to have lower overall metal concentrations in their above-ground parts than do rosette-type plants, due to a dilution effect. In contrast with the trends observed for the other metals studied (St-Cyr and Campbell 1994), Pb bioconcentrates more in *P. richardsonii* and *Heteranthera dubia* (stem-bearing plants) than in *V. americana* (rosette-type plant). Similar results were reported by Crowder *et al*. (1989) for the Bay of Quinte, Lake Ontario, where *Myriophyllum* sp. accumulated appreciable Pb (up to 9.6 µg/g) at sites where this metal was undetectable in *V. americana* (< 1 µg/g). In their laboratory studies, Welsh and Denny (1979) found only minimal translocation of Pb from roots to shoot for *Potamogeton* spp. However, shoot uptake of dissolved Pb from the surrounding water presumably does occur (Welsh and Denny 1979; Knowlton *et al*. 1983). Perhaps the greater surface to volume ratio of *Potamogeton richardsonii* and *Myriophyllum* sp. relative to *Vallisneria americana* influences the accumulation of Pb more than the other metals; high surface to volume ratios are known to favour metal uptake from water (Franzin and McFarlane 1980).

In order to reduce the variability while sampling macrophytes for monitoring purposes, it would be easier, if possible, to sample rosette-type species as they are also easier to clean. Alternatively, plants such as *Eleocharis* sp. for example, could be selected, where the stem, which is the photosynthetic organ of the plant, dominates the above-ground parts (the leaves are very reduced).

#### 1.4.2.2 Seasonal and within-site variability

Metal concentrations in the above-ground parts of submerged plants tend to vary throughout the growing season (Gommes and Muntau 1981; Lyngby and Brix 1982; Guilizzoni 1991; St-Cyr and Campbell 1994). In general, spring growth is characterized by mobilization of carbon reserves and mineral nutrients in the rhizome or overwintering buds, and by a rapid uptake of nutrients (and metals). At this time, accumulation of mineral nutrients by aerial tissues exceeds that required to sustain the current growth. As the aerial tissue becomes self-supporting, mobilization from the rhizome gives way to translocation of carbon compounds and mineral nutrients from leaf tissue to the developing reproductive organs and once again back to the rhizomes for winter storage. During the active period of growth, the metal concentrations in the aerial tissues normally decline, also due to a dilution effect because of the rapid production of organic matter. During senescence, metal concentrations build up again due to the breakdown of tissues and attachment of detrital organisms which increase surface area and binding sites for metals. It follows that in order to compare metal concentrations in plant tissues from different stations, plants must be collected from the same period of the year. Often, the preferred sampling time is at the end of the summer (August), when aquatic plants have reached their full seasonal growth.

Within-site variability in metal concentrations of aquatic plant tissues has been recorded by several researchers. Coefficients of variation of the order of 10-30% seem to be frequently reported, but sometimes variation is higher (Campbell *et al*. 1985; St-Cyr and Campbell 1994). Campbell *et al*. (1985) reported that possible explanations for this variability include the existence of distinct microhabitats within the sampling site, the collection of specimens of differing age, and the sampling of plants at slightly different stages in their growth cycle. Campbell (in Tessier *et al*. 1982) suggested the preparation of a composite sample from several specimens at a given site in order to minimize within-site variability.

### 1.4.3 Biochemical indicators

Besides total metal accumulation by aquatic plants in contaminated freshwater environments, there is a very interesting area of research that has not yet been thoroughly investigated on biochemical indicators of stress. Only part of the metals assimilated by a plant will be phytotoxic, namely the fraction which interferes with cellular metabolism. The metal bound to the cell wall or accumulated in vacuoles has no physiological effect. Simple chemical analysis cannot distinguish between these fractions. Biochemical responses to chemical effects are based on the principle that all toxic effects begin with a relation between the toxic chemical and some biochemical receptor in a living organism. Toxic effects on ecosystems begin with these chemical reactions in individuals. Detection and measurement of these chemical reactions in individuals should provide specific and sensitive diagnostic tools that give early warning, since the chemical reactions are unique and precede effects at higher levels of organization (NRCC 1985).

Two of these biochemical indicators, phytochelatin induction and increased enzyme activity, have been fairly well studied in terrestrial systems, particularly those affected by mine activities. Only a brief description of these two biochemical indicators will be provided here, but they offer a very interesting area of research for aquatic environments.

#### 1.4.3.1 Phytochelatins or plant metal-binding proteins

Phytochelatins, or metal-binding proteins of the plant kingdom, can be considered the plant equivalent of metallothionein in the animal kingdom (Couillard 1997); they both complex metals in the cell cytosol, but they are different both in structure and biosynthesis. Mostly studied with terrestrial plants, and often in cell cultures, these proteins are now considered to be ubiquitous in the plant kingdom, and have aroused considerable interest. Many interesting review papers are available on the subject (Grill *et al.* 1990; Rauser 1990; Reddy and Prasad 1990; Steffens 1990).

Phytochelatins are low molecular weight linear polymers, having the general formula:

$$(\gamma\text{-Glu-Cys})_n\text{-Gly}$$

where Glu=glutamic acid, Cys=cysteine, Gly=glycine, and n varies from 2 to 11. An enzyme, phytochelatin synthase, catalyzes their biosynthesis. Sulfhydryl groups of the cysteine give to this protein a high affinity for metals such as $Cd^{2+}$, $Cu^{2+}$, $Zn^{2+}$, $Ag^{+}$, $Pb^{2+}$ and $Hg^{2+}$, and phytochelatins are induced by these metals, particularly by $Cd^{2+}$. However, phytochelatin can also be normally present in plant cytosol to balance the physiological availability of essential metal ions such as $Cu^{2+}$ and $Zn^{2+}$, and provide a mechanism for homeostasis of these ions, and may be also involved in the cell sulfur metabolism.

Phytochelatins were reported to be induced in contaminated environments (in the laboratory, in cell cultures growing in a contaminated medium; in the field, in roots of plants growing on contaminated soils) while controls growing in an uncontaminated environment revealed no synthesis of phytochelatins (Grill 1987; Gawel *et al*. 1996). The report of the occurrence of these metal-binding peptides in contaminated environments supports a role of phytochelatins in metal detoxification in plants. Efficient sequestration of toxic metal ions reduces or prevents deleterious effects of the ions by physiological inactivation. Several lines of evidence support this view of a detoxifying function of phytochelatins. If plants respond to metal stress by inducing these metal-binding proteins, then these proteins could serve as «indicators» of the presence of bioavailable metals in the environment.

There is no reason to believe that aquatic plants will react differently than terrestrial ones, which are reported to synthesize phytochelatins while exposed to sub-lethal concentrations of metals as Cd and Cu, in the field. The presence of a Cd-binding complex was reported in the roots of *Eichhornia crassipes*, a free-floating aquatic plant, while growing in a $Cd^{2+}$-containing medium (Fujita and Kawanishi 1986). However, Vachon (1995) was unable to detect the presence of such proteins in *Vallisneria americana* and *Myriophyllum spicatum*, both submerged plants, collected in the St. Lawrence River. It would be very interesting to test if rooted submerged plants growing in contaminated sediments due to past or current mine activities reveal the presence of such proteins in their tissues.

#### 1.4.3.2 Enzyme induction

Uptake of phytotoxic amounts of metals by plants can also result in inhibition of several enzymes (particularly those involved in photosynthesis), and in an increase in activity (=induction) of others, due to the interference of metals with metabolic processes in the plant.

Enzyme induction is supposed to play an important metabolic role under conditions of metal stress. Peroxidase induction, for example, is a general response of higher plants to uptake of toxic amounts of metals, likely related to oxidative reactions at the biomembrane;

several enzymes involved in intermediary metabolism might be stimulated to compensate for metal-sensitive photosynthetic reactions (Van Assche and Clijsters 1990a). The induction of enzymes, and metal-specific changes in the isoperoxidase pattern, can be used as diagnostic criteria to evaluate the phytotoxicity of soils, contaminated by several metals, for higher terrestrial plants. A biological test system was elaborated by Van Assche and Clijsters (1990b). The isoperoxidase patterns in leaves and roots of *Phaseolus vulgaris* grown under controlled conditions in contaminated and control soil samples was obtained, and were used to quantify and map the potential phytotoxicity of soils surrounding an old closed Zn smelter, contaminated by several metals. Byl and Klaine (1991) and Byl *et al*. (1994) measured peroxidase (POD) activity in *Hydrilla verticillata*, a rooted submerged plant, exposed in the laboratory to sublethal concentrations of several toxins and metals such as $Cu^{2+}$ and $Cd^{2+}$ and reported a significant increase in POD activity following exposure to the metal. The increase in POD activity corresponded to increasing concentrations of toxins. They proposed that measuring POD activity has great potential as a biochemical indicator of sublethal stress in this plant.

Rabe *et al*. (1982) exposed apical shoots of *Elodea canadensis*, in the laboratory, to different Cu levels, and observed that the activities of the enzymes isocitrate dehydrogenase and glutamate dehydrogenase increased under slight or moderate stress, which can indicate a plant injury before visible damage occurs. Under longer exposure and higher Cu levels, the enzyme activities decreased markedly.

Until now, most of the studies about peroxidase activities in response to pollution stress have been done in the laboratory, with organic contaminants and herbicides (Sprecher and Stewart 1995; Sprecher *et al*. 1993); a field study with aquatic plants affected by pulp and paper mill effluents has also been recorded (Roy and Hänninen 1994; Roy *et al*. 1992). The main purpose of this method of measuring peroxidase activities is to rapidly and economically monitor the levels of toxic waste released into the aquatic environment. Tolerance to water pollution was consistently related to high peroxidase activities.

## 1.5 CONCLUSION

In a first general survey of regions affected by mine activities, plant communities inhabiting water bodies can be listed. The total absence of macrophytes from water bodies where they would normally be found can indicate general pollution (metal toxicity, acid conditions...) due to mining or smelting activities. The presence of only some indicator species (which are tolerant to acid conditions such as bryophytes, Isoetids, *Eleocharis* sp., *Eriocaulon* sp., *Juncus* sp.) can be the result of mining activities if other species found in reference sites are absent. However, isoetid and plant species having leaves in rosette form such as *Eriocaulon septangulare* are characteristic vegetation of oligotrophic soft-water lakes and the presence of these plants is not necessarily the result of perturbations due to mining activities. Comparison with a healthy environment is the basis of the method, but the ideal reference site which must be similar in all respects, except for the presence of metal contamination and of acidity coming from mining activities, can be hard to find.

Rooted macrophytes possess several of the attributes of an «ideal» biomonitor. Among others, they are sedentary, visible to the naked eye, easy to collect and to handle, they concentrate metals in their tissues and reflect the environmental contamination. Sediment seems to represent an important source of metals for rooted submerged plants. The total metal concentration in the sediment is not a good predictor of metal concentrations in plant tissues which reflect the free-metal ion concentrations in the interstitial water. Roots are better bioindicator organs than shoots to reflect the sediment metal contamination. Metals in above-ground parts of the plant can come from direct uptake from the surrounding water (particularly if metal concentrations are high in the water column) and/or from acropetal translocation from the roots/rhizomes. Both above- and below-ground parts of the plants must be adequately cleaned before metal determination. Emergent plants and plants with floating leaves can be directly affected by atmospheric deposition of metals. It is recommended to collect composite samples of a species at a given site in order to reduce within-site variability, and to complete a sampling campaign during a particular period of the year, to avoid seasonal variations.

Biochemical indicators of stress due to metals, such as phytochelatins and enzyme induction (peroxidase activities), await further development but appear very promising as biomonitoring methods.

## 1.6 RESEARCH NEEDS

The relationships between metal concentrations in the tissues, cleaned from external contamination, of rooted submerged macrophyte species and those bioavailable in the sediment interstitial water must be investigated by field sampling over a contamination gradient. The free-metal ion concentrations in the oxic layer of the sediment, at the interface sediment/water, must be determined for all metals which are of interest to the mining industry.

Biochemical indicators of stress in plants, such as phytochelatins and enzyme induction, particularly peroxidase activity, deserve as much attention as those measured in animals, such as metallothionein and hepatic mixed function oxidase activity. Their use as biomonitoring tools appears promising.

# 2. PHYTOPLANKTON

by

Antonella Cattaneo

## 2.1 OVERVIEW OF BIOMONITORING USING PHYTOPLANKTON

Several reasons suggest the use of phytoplankton for biomonitoring. Because algae are at the base of the trophic chain of lakes and many rivers, an impact on these communities will have broad repercussions on the functioning of the entire system. Many environmental problems, like cyanophyte blooms, result directly from changes in the algal community. Rapid reproductive rates make algae very responsive to changes in water quality. Siliceous remains of diatoms and chrysophyceae can be used in paleolimnological studies to detect past pollution episodes and to establish the composition of pre-pollution communities.

Several methods have been developed for use of phytoplankton to monitor lakes and rivers (Whitton 1984; Cairns *et al*. 1993; Whitton and Kelly 1995). Biotic indices and indicator species have been described mostly for organic pollution and acidification. For metal contamination, the methods address either the entire community or the single species and fall broadly into 6 categories: 1) cell accumulation of the contaminant, 2) changes in biomass or production of the entire community, 3) shifts in community composition measured as diversity, changes from sensitive to tolerant species, or alterations of size distribution, 4) development of genetic tolerance, 5) physiological indicators including changes in pigment composition, enzyme inhibition (e. g. alkaline phosphatase), and phytochelatin induction, 6) morphological indicators like deformities and size changes.

None of these methods are as routinely applied as, for example, the biotic indices based on benthos. In the studies at mine sites, only a few of these methods have been attempted. A thorough field validation is necessary for new approaches that appear promising.

## 2.2 CASE STUDIES

### 2.2.1 Phytoplankton and biomonitoring at mine sites

#### 2.2.1.1 Buttle Lake, Vancouver Island, British Columbia

Buttle Lake is the largest (42,000 ha) and deepest (mean depth 45 m) lake in the Campbell River watershed situated in the central part of Vancouver Island. Buttle Lake drains through a short channel into Upper Campbell Lake. The Campbell system continues through Lower Campbell Lake, John Hart Lake, and the Campbell river that flows into the sea. In 1966, a copper-lead-zinc mine (Westmin Resources Ltd.) began operation near the south end of Buttle Lake. Tailings were discharged into the lake via a submerged outfall because mountainous terrain made land disposal difficult. Peak concentrations as high as 370 µg/L Zn, 40 µg/L Cu, 25 µg/L Pb and 3.6 µg/L Cd were reached in 1981. Concern over the deterioration of the Campbell river system, an important habitat for salmon, prompted a multidisciplinary study on the effects of mining on benthos, phytoplankton, periphyton, and zooplankton and on metal accumulation in fish (Roch *et al*. 1985). For phytoplankton, it was possible to compare data collected in the late 60's prior to or at the onset of the mine operation with those collected in 1980-81 at the peak of the metal pollution. There was a shift in composition from the association of *Rhizosolenia*/*Tabellaria*/*Ceratium*/*Peridinium* typical of the region's oligotrophic lakes to an anomalous one dominated by *Navicula cryptocephala*, *Synedra acus*, *Synedra filiformis*, *Cyclotella bodanica*, and *Cyclotella glomerata*. Previously abundant diatoms intolerant of metals such as *Tabellaria fenestrata*, *Tabellaria flocculosa*, and *Asterionella formosa* had virtually disappeared in Buttle Lake by 1980 (Austin and Monteanu 1984). *Rhizosolenia* and *Tabellaria* were, however, still abundant in the downstream lakes (Lower Campbell and John Hart), while *Asterionella* remained abundant only in Quinsam Lake located in a nearby unpolluted watershed. At the class level, Chlorophyceae, Chrysophyceae, and Pyrrophyceae were better represented in 1960's samples while Bacillariophyceae's abundance and number of species increased in 1980-81 samples; cyanophyceae remained unchanged. In the south basin of Buttle Lake directly affected by the effluent, the number of species declined from 51 in the 1960's to 39 in 1980's; the Shannon-Weaver diversity index decreased over the same period from 1.03 to 0.71. Temporal changes were less important at downstream stations. However, there was an increase in phytoplankton density between the 1960's and the 1980's probably as consequence of decreased grazing by zooplankton that was severely reduced by metal inputs (Roch *et al*. 1985). Because production measurements prior to the pollution were unavailable, comparisons could be made only along the downstream metal gradient. In summer 1981, production was significantly lower at the two locations closest to the mine from April to July. However, when considering the entire 6 month ice-free period, the most contaminated site did not differ significantly from the downstream sites except for the reference site at Quinsam Lake.

Various aspects make the Buttle Lake example particularly instructive. The lake remained neutral throughout the study (pH 6.5-7.3) and its oligotrophic status was not altered by mining operations at least until the early 80's. Consequently, metal effects were not confounded like in many other studies of mine sites by co-occurring increases in trophy and acidity. These effects should have been accentuated because hardness was low (20-30 mg/L $CaCO_3$) and some metals are more toxic in soft water. Another interesting feature is that it has been possible to monitor the partial recovery of the system after implementation of remedial measures in 1983 that reduced the metal loading by 80%. Deniseger *et al*. (1990) followed the response of phytoplankton, zooplankton, and fish during the 3 year transition from high to low metal concentrations. In the phytoplankton, after a transition phase in which *Rhizosolenia* became extremely abundant forming practically a monoculture, previously dominant metal sensitive species as *Tabellaria* and *Asterionella* have started to reappear. The bloom of *Rhizosolenia* was probably the result of increased nutrients and in particular an increase of the N/P ratio following sewage and land disturbance related to increased mine production. *Rhizosolenia*, a species previously described as intolerant of pollution, reached bloom conditions at metal concentrations that, while reduced compared to the peak values of 1981, were still well above background levels. The capacity of this alga to bloom in conditions that previously led to its demise, is a good example of development of metal tolerance in algae.

2.2.1.2 Sudbury, Ontario

In this area are concentrated many mines and smelters, some of which have been in operation for the last 80 years. Many studies have addressed the dramatic effects of such intensive
mining on the environment. Because of the size of the smelting operations, the contamination of lakes and rivers by atmospheric deposition of acid and metals covers a large radius around Sudbury.

*Clearwater Lake*

The phytoplankton community of this severely impacted lake has been monitored since 1973 and compared with that typical of lakes similar in trophy and morphometry but unpolluted (Yan 1979). This lake, located 12 km south of the Cu-Ni smelting complex in Copper Cliff, near Sudbury, had elevated concentrations of Cu (90 to 110 µg/L) and Ni (270 to 290 µg/L) and had been acid (pH ~ 4.3) for at least the last 20 years. Despite this severe pollution, phytoplankton biomass, averaged over the years, was similar to that of nutrient-poor lakes in Ontario with pH levels between 5.5 and 7.0. The number of genera in Clearwater Lake was on average only half that in a reference morphometrically similar lake (Blue Chalk Lake) that had not been acidified. However, the numbers of diatom and desmid taxa, and possibly dinoflagellate taxa, were not reduced. While lakes of similar trophy of this region are dominated by chrysophytes, Clearwater Lake communities were dominated by dinophytes, in particular *Peridinium incospicuum* that represented between 30 and 55% of the average ice-free biomass. Analyzing by multiple regression data for thirteen other lakes, variously impacted by acid and metals, abundance of *P. incospicuum* correlated to acidity rather than to metal concentrations.

*Baby and Alice Lakes*

The phytoplankton of two other lakes of the Sudbury area has been studied in depth. Baby and Alice lakes are immediately adjacent to the Coniston smelter, which operated from 1913 to 1972 when it was permanently closed. In 1968, these two lakes were so severely impacted that only a few cells of metal tolerant *Chlorella* could be filtered from their waters (Hutchinson and Stokes 1975; Stokes *et al*. 1973) that were practically devoid of zooplankton and fish. The small but deep Baby Lake had a low pH (4.0) and extremely high concentrations of Cu (to 800 µg/L) and Ni (to 3,200 µg/L). The slightly larger and shallower Alice Lake was less acidic (pH 5.9 - 6.3) but had even higher concentrations of Ni (to 7000 µg/L). Once the local smelter closed, water quality improved (Hutchinson and Havas 1986) and, by 1986, the pH of the two lakes (6.1-6.8 for Baby and 6.9-7.4 for Alice) was similar and close to neutrality, but Ni concentrations in Alice Lake remained more elevated (1,300 µg/L) than in Baby Lake (400 µg/L). Compared with 1970-72, phytoplankton biomass had increased greatly in both lakes (79 µg/L in Baby and 640 µg/L in Alice), even if it remained low compared to lakes of similar trophy. Diversity had also increased: 19 and 37 species of phytoplankton were described in Baby and Alice Lake respectively (Havas *et al*. 1995). The phytoplankton community of Baby Lake was dominated (94% of cell counts) by Chrysophyceae, in particular *Dinobryon bavaricum*, while in Alice Lake, Bacillariophyceae represented 84% of cell counts and *Rhizosolenia eriensis* was the dominant taxon. Further improvements in the water quality were observed in the early 90's. Both lakes were neutral but Ni remained high in Alice Lake (1,200 µg/L). In Baby Lake, the chrysophyte *Kephrion* accounted for 69% of the total cell counts, but *Dinobryon*, *Peridinium*, and *Chlorella* were also frequent. A substantial change in composition was instead described in Alice Lake where *Cosmarium* dominated the community with 87% of all cells while the previously dominant Bacillariophyceae accounted only for 6% (Havas *et al*. 1995). The biological recovery was slower than chemical recovery and was characterized by unbalance and dominance of few species.

Additional insights in the ability of phytoplankton to monitor recovery from anthropogenic stresses can be gleaned from other studies in Sudbury area lakes mainly focused on acidification. In Bowland and Trout Lakes, experimental liming and consequent pH increase (from 4.9 to 6.8 and from 5.8 to 6.5 for Bowland and Trout respectively) were accompanied by a rapid increase in phytoplankton diversity and a shift away from dominance of cyanophytes to dominance of the prymnesiophyte *Chrysochromulina breviturrita*; biovolume and chlorophyll remained unchanged (Molot *et al*. 1990).

*Multi-lake surveys*

Besides these studies centered on particular lakes, the expansion of the pollution over a large radius has allowed comparative studies of entire phytoplankton assemblages (Nicholls *et al*. 1992) and sedimentary chrysophytes and diatoms ( Dixit *et al*. 1989, 1991) in many lakes along a gradient in acidification and metal pollution. In these studies, canonical correspondence analysis was used to infer the importance of various limnological variables on the algal composition. Nicholls *et al*. (1992) found that community variation, both spatial (across 111 lakes) and temporal (over mid 1970's - mid 1980's in 7 lakes), were mostly explained by factors related to acidity and trophy. Acidity was strongly related to number of species that increased with pH or with alkalinity; biomass, in contrast, was uncorrelated with acidity. Aluminum, resulting from weathering of the watershed by acid precipitation, was the only metal that was related to species composition; more taxa were present at low Al concentrations. However, it was not possible to differentiate between the effect of Al and the effect of acidity because these two variables tended to co-vary.

In the 72 lakes studied by Dixit *et al*. (1989, 1991), located within 100 km of Sudbury, lakewater pH was the most important variable related to sedimentary chrysophyte and diatom assemblages. However, chrysophyte distribution was also related to concentrations of Cu and of associated metals. For example, *Chrysodidymus synuroides*, *Synura echinulata*, and *Mallomonas hindonii* predominated in lakes that had high metal concentrations. Diatom distribution was also in part related to metal concentration (Al, Cu, and Ni). *Pinnularia hilseana*, *Eunotia tenella*, *E. exigua*, and *Frustulia rhomboides* v. *saxonica* predominated in the most impacted lakes. These paleolimnological methods were also able to detect the recent recovery from acidification and metal contamination. In the superficial strata of the cores corresponding to the onset of emission reduction from the Sudbury smelters, chrysophyte and diatom assemblages were more similar to those observed prior to contamination (Dixit *et al*. 1992).

2.2.1.3 Other mine sites

*Meg, Keg, and Peg lakes, Canadian subartic*

These three small, shallow, and eutrophic lakes are located near the north shore of Great Slave Lake, Northwest Territories. Meg Lake, at the head of the system, receives waste from a gold mine that began operation in 1938. These mine effluents raised considerably the concentrations of As (to 3,000 µg/L), Pb (to 650 µg/l), cyanide (to 600 µg/L), and Cu (to 650 µg/l) in Meg Lake. Although the levels of As and Pb in the other two lakes are roughly similar to those of Meg Lake, cyanide and Cu usually fell below detectable limits. Despite these different degrees of contamination, phytoplankton biomasses (150-200 µg/L) were similar among the three lakes (Moore *et al*. 1979). However, these biomasses were much lower than those usually observed in lakes of the Precambrian Shield (1,000-3,000 µg/L; Kling and Holmgren 1972). *Chlamydomonas lapponica* was dominant in spring in all lakes. The summer dominant was *Aphanothece microspora* in Meg Lake, while in Peg and Keg lakes, *Oocystis parva* dominated the summer community. These species are widely distributed and abundant in unpolluted lakes of the arctic and subartic and were not previously associated with metal contamination. Because of the high level of metals in these lakes, these algae must have the capacity to adapt to this pollution. It should be taken into account that the high hardness of these lakes may have reduced metal toxicity. The number of species was lower in Meg (16) than in the two less polluted lakes (26-27).

*Rabagao River's reservoirs, Portugal*

This chain of three mountain reservoirs is located in North Portugal, in a predominantly granitic zone. The waters are characterized by very low alkalinity (5.3-5.4 mg/L $CaCO_3$), neutral pH (6.9-7.5) and low nutrient levels. The reservoirs were built between 1951 and 1964 mainly for hydroelectric purposes. A copper pyrite and tungsten mine discharges its effluents in the second reservoir, Venda Nova. The effects of the mine effluent on the phytoplankton communities were studied monthly during 1981-1982 at 4 stations differently impacted by Cu. Phytoplankton density and biomass were significantly depressed at the most contaminated site (Oliveira 1985). In contrast, no inhibition of photosynthesis was detected at that site since primary productivity per unit chlorophyll was not significantly different among reservoirs (Cabeçadas and Brogueira 1987). There was a shift in dominant species among the reservoirs (Oliveira 1985). Phytoplankton communities in the upper reservoir, that served as a control (4 µg/L Cu), were dominated by *Crucigenia tetrapedia*, *Quadrigula closteriodes*, *Tabellaria flocculosa*, and *Melosira distans*. In Venda Nova, which received mine wastes, and in the downstream reservoir, phytoplankton was dominated by the desmid *Cosmarium tinctum*. At the most polluted station (to 49 µg/L Cu), Bacillariophycae was the algal group the most depleted while Chrysophyceae was the least affected. The number of species was significantly lower at the most impacted site, but Shannon Weaver and evenness indices were not significantly different among sites.

### 2.2.2 Phytoplankton in non-mine-related biomonitoring studies

While most experiments with metals are short term and unable to simulate the response of lakes and rivers to chronic metal pollution, in the two following examples natural populations were exposed to a more realistic long-term metal enrichment. Another example of phytoplankton response to chronic metal pollution is offered by Lago d'Orta.

#### 2.2.2.1 MELIMEX experimental study, Switzerland

This experiment was conducted for almost two years (March 1977 to July 1978) using three limnocorrals (diameter 12 m, volume 1,130 $m^3$) installed in Lake Baldegg, a highly eutrophic lake in central Switzerland. The purpose of this study was to examine the long-term effects of the legally maximum tolerated metal load on phytoplankton, zooplankton, benthos, and bacterial activity. Two of the limnocorrals were dosed with Hg, Cu, Cd, Zn, and Pb, and led to concentrations approximating the legally tolerated limits (0.16 µg/L Hg, 6.4 µg/L Cu, 2.2 µg/L Cd, 196 µg/L Zn, 8.3 µg/L Pb). The third limnocorral with metal concentrations similar to those in the lake (1.3 µg/L Cu, 0.11 µg/L Cd, 19.6 µg/L Zn, 0.41 µg/L Pb) was used as a control. Initially, increased metal concentrations depressed the photosynthetic activity of phytoplankton, resulting in lower chlorophyll in the metal-loaded limnocorrals (Gächter and Máreš 1979). Later, shifts in phytoplankton species composition towards taxa more tolerant and decreased zooplankton grazing counteracted the initial depression of phytoplankton growth. On a long-term basis, elevated metal concentrations did not decrease production or biomass (as chlorophyll). Seasonal shifts in species composition were often very sharp and sudden, making the interpretation of the results rather difficult. However, the authors identified 7 groups as consistently more abundant in the treatments than in the control: various cyanophytes (but not *Aphanizomenon flos aquae*), various diatoms (but not *Fragilaria* spp. and *Cyclotella* spp.), *Ankistrodesmus falcatus*, *Botryococcus braunii*, *Chlorella pyrenoidosa*, *Raphidium* spp., and *Schroederia setigera*. There was a slight tendency towards higher species number in the control compared to metal-loaded limnocorrals. However, this tendency did not result in depression of the Shannon-Weaver diversity index that is more affected by the evenness of allotment of individuals among species than by the number of species.

2.2.2.2 Lake 382, ELA, Ontario.

Cadmium was experimentally added from 1987 to 1992 to Experimental Lake Area (ELA) Lake 382 to reach a maximum concentration in the final year of 0.185 µg/L. This concentration is close to the Canadian Water Quality Guideline for soft water lakes. It is much lower than concentrations usually used in metal contamination studies but is 100 times higher than the lake background level. The goal of the study was to measure the effects of a low concentration of a toxic metal on a natural ecosystem. The effects on the phytoplankton population were assessed by comparisons with 9 years of premanipulation data for the same lake and data for 2 nearby reference lakes (Findlay *et al*. 1996). Small changes in photosynthesis and biomass over the experiment were related to nutrient input. Diversity, species composition and size distribution were not affected by the Cd addition. The abundance of species that have been considered sensitive to Cd (*Rhabdogloea gorskii*, *Chlamydomonas* spp., *Elakatothrix gelatinosa*, *Dinobryon bavaricum*, and *Synedra acus*) did not change significantly. Shifts in composition had been observed in enclosures in nearby Lake 223 in 1977 but the Cd concentrations were 1, 3, 10, and 30 µg/L (De Noyelles *et al*. 1980). Similarly, significant decreases in photosynthesis were documented in studies with Cd concentrations in excess of 1 µg/L (Takamura *et al*. 1989).

Experiments at ELA have also examined the effects of acidification/recovery on the phytoplankton. Because acidification is often associated to mining operations, these results could provide useful insights into the monitoring potential of phytoplankton. Experimental acidification of Lake 223 from pH 6.7 to 5.1 decreased species diversity, increased biomass and resulted in a shift from chrysophytes to dinoflagellates. During recovery, these trends were reversed after pH reached 6.0. Diversity, biomass, taxonomy, and size structure resembled closely those of communities in reference lakes (Findlay and Kasian 1991, 1996).

2.2.2.3 Lago d'Orta, Northern Italy.

This deep subalpine lake (maximum depth 143 m) was dramatically polluted in 1926 when a Rayon factory started to discharge Cu and ammonium, decimating the phytoplankton, zooplankton, fish, and even bacteria. Highest concentrations of Cu were measured in the 50's (to 110 µg/L). Although Cu discharge from the factory was significantly reduced in 1958, loading of Cu, Zn, Ni, and Cr from electroplating industries became significant. The biochemical oxidation of ammonium acidified the lake (to pH 4) from 1970 onwards. After an initial almost complete collapse, phytoplankton showed ephemeral appearances and bursts of different species in very poor communities. Among the species reported in the 60's were *Oocystis*, *Oscillatoria limnetica*, *Coccomixa minor*, *Microcystis aeruginosa*, and *Scenedesmus armatus*. By the 80's (Cu: 36 µg/L, Cr: 2 µg/L, Ni: 17 µg/L, Zn: 56 µg/L) the phytoplankton was still an anomalous "chlorophycean plancton" dominated by *Coccomixa* and other small-sized algae; diatoms, dominant in nearby lakes, were very rare and mainly represented by *Achnanthes minutissima* (Bonacina *et al*. 1988). In 1989, the lake was limed and pH increased (6 to 7) while metal concentrations decreased due to precipitation (Cu concentration in the epilimnion: 5-15 µg/L). The response to the liming was striking; after only 1 month, diatoms became dominant. *Achnanthes minutissima* and *Synedra acus* dominated in spring while *Rhizosolenia eriensis*, a species that had disappeared since the onset of the pollution in 1926, became the dominant in the fall peak. There was a three-fold increase in number of species in the two years following liming. These dramatic changes cannot be entirely ascribed to metal abatement for there was a contemporaneous reduction in acidity and ammonium in the water.

## 2.3 CRITICAL EVALUATION OF THESE AND OTHER POTENTIAL MONITORING METHODS

### 2.3.1 Accumulation

Phytoplankton are able to take up metals from water, to bind them on their surface, and to produce exudates with metal-complexing properties. Reduction of dissolved metal concentration by phytoplankton has been observed not only in culture but also in lakes, rivers, oceans (Sigg 1987; Slauenwhite and Wangersky 1991). Metals adsorbed on the algal surface are removed from the water column when these cells sediment; metals are complexed by extracellular ligands produced by the algae. While these processes are important for mitigating the potential toxic effects of metals to aquatic organisms, their utilization as monitoring tools is problematic. Laboratory studies of algae, under controlled conditions, have demonstrated that metal absorption and uptake depend on the metal's speciation and the medium pH (Campbell and Stokes 1985; Harrison *et al*. 1986; Schenck *et al*. 1988). In presence of high Cu concentration, cell accumulation is prevented by production of exudates (Xue and Sigg 1990). As a result, algal metal accumulation is not readily related to external concentration (Cain *et al*. 1980). However, Denny and Welsh (1979) observed different Pb content in plankton from three lakes in the English Lake District variously affected by old lead mines in their catchment.

For most contaminants, tissue accumulation is better measured in organisms high in the trophic chain. This does not appear to be the case for metals, where biomagnification has not been observed. Algae tend to contain higher metal concentrations in polluted and unpolluted situations than the next member in the food chain (Gächter and Geiger 1979; Forstner and Wittman 1981; Prahalad and Seenayya 1989; Sanders *et al*. 1989).

### 2.3.2 Density, biomass, and production

In short-term experiments, addition of metals to algal cultures or natural phytoplankton usually result in a decrease of standing crop and inhibition of photosynthesis (Blinn *et al*. 1977; Côté 1983; Takamura *et al*. 1989; Havens 1994; many more examples in review articles of Rai *et al*. 1981 and De Filippis and Pallaghy 1994). However, in long-term experiments (Thomas and Seibert 1977; Gächter and Máreš 1979) and in the case studies that we have examined, where metal pollution was severe but chronic, significant differences in production and biomass between impacted and reference sites were not always observed. After an initial depression, tolerant species eventually replace the sensitive ones achieving similar levels of biomass and production as control systems. In some instances (Thomas and Seibert 1977; Gächter and Máreš 1979; Austin and Munteanu 1984), biomass can even be more elevated in impacted systems due to the toxic metal effect on zooplankton and consequently reduced grazing pressure. Clearly, when the pollution is extreme, as in the example of Baby and Alice lakes at the peak of the acid and metal contamination (Havas *et al*. 1995), the capacity of the communities to adapt is exceeded.

There are some problems in interpreting significant differences based on few samplings. As Yan (1979) points out in his study of Clearwater Lake, biomass may vary substantially over very short periods and more than two-fold among years: any difference in biomass between lakes must be carefully examined before it is considered significant. This variability demands a strong sampling effort ideally extended over many years and various reference lakes. Because biomass and density may adapt and recover rapidly following the onset of contamination, Schindler (1987) has proposed that changes in community structure (taxonomic composition, diversity, etc...) may be more sensitive indicators of ecosystem stress.

### 2.3.3 Community structure

#### 2.3.3.1. Diversity

Various indices have been proposed to describe the diversity of a community (for review see Cairns *et al*. 1993). The simplest is the number of species per sampling, but the most commonly used is the one proposed by Shannon and Weaver (1949) that considers both the species richness of a community and the degree of dominance or evenness among these species. The proposed relationship between diversity and environmental stress has not always been confirmed (Stevenson 1984) but it seems to hold better for metal pollution than for other stresses. Usually, diversity decreases with increasing concentrations of metals (Thomas and Seibert 1977; Say and Whitton 1980). In the case studies considered here, a more or less drastic decrease in species number was observed in the metal impacted sites. The only exception was Lake 382 where metal contamination was very low (Findlay *et al*. 1996). However, the Shannon and Weaver index was not always sensitive to the pollution changes (Gätcher and Máreš 1979; Oliveira 1985) because the species evenness was not affected. Number of species and probably number of genera could be useful parameters for biomonitoring but they require a technical specialist (a little less if the analysis is not extended beyond the genus level). Quantitative samples are not necessary but the effort of counting should be similar for all samples and should continue until new species or genera are rarely encountered.

#### 2.3.3.2 Indicator species

The study of algal community response to pollution has been traditionally based on indicator species (Palmer 1959) but these studies have mostly addressed organic pollution. From this examination of the literature, a consistent pattern of tolerance or sensitivity to metal pollution can be found for some species. *Chlorella*, *Cosmarium*, *Scenedesmus*, *Gomphonema parvulum* and *Peridinium* appear consistently in the group of the tolerant species while *Asterionella formosa*, *Tabellaria*, *Crucigenia tetrapedia*, and *Rhodomonas* are always reported as sensitive to metal pollution. Strikingly, *Rhizosolenia* is dominant in three examples of systems undergoing recovery after severe metal pollution (Deniseger *et al*. 1990; Bonacina *et al*. 1992; Havas *et al*. 1995) located as far apart as British Columbia, Italy, and Ontario. This broad geographical distribution is observed also for other species which span, in our examples, from Portugal to the Northwest Territories. However, there are also several species that behave differently in different situations, like *Cyclotella*, various species of *Dinobryon*, *Elakatothrix*, and *Oocystis* (see Table 2.1). A possible complication for the interpretation of species composition is the often reported (see section below on genetic tolerance) development of tolerance to metals so that a taxon described as sensitive can produce strains capable of thriving under severe pollution.

At the class level, there are controversial results regarding the variation of tolerance to metals among algal groups. Following the landmark studies of Foster (1982) and Whitton (1984) in British streams differently affected by mining, it was proposed that cyanophytes are the most sensitive class while chlorophytes are apparently more tolerant to metal pollution. This conclusion is probably more applicable to benthic algae than to phytoplankton. This tendency was partly confirmed in our examination of the literature with only green algae surviving in the more drastically polluted Sudbury lakes (Stokes *et al*. 1973) and becoming dominant in the metal

**Table 2.1** Resistant (R), sensitive (S) or indifferent (I) phytoplankton species to metals.

| Species | Tolerance to metals | Reference |
|---|---|---|
| *Ankistrodesmus falcatus* | R | Gächter and Máreš 1979 |
| *Aphanizomenon flos-aquae* | S | Gächter and Máreš 1979 |

| | | |
|---|---|---|
| *Aphanothece microspora* | R | Moore *et al*. 1979 |
| *Asterionella formosa* | S | Austin and Munteanu 1984 |
| | S | Reinke and De Noyelles 1985 |
| *Botryococcus braunii* | R | Gächter and Máreš 1979 |
| *Ceratoneis arcus* | I | Oliveira 1985 |
| *Chlamydomonas lapponica* | R | Moore *et al*. 1979 |
| *Chlorella pyrenoidosa* | R | Gächter and Máreš 1979 |
| *Chlorella vulgaris* | R | Loez *et al*. 1995 |
| *Chlorella* sp. | R | Stokes *et al*. 1973 |
| *Chrysochromulina parva* | R | Wängberg 1995 |
| *Chrysospherella longispina* | R | Wängberg 1995 |
| *Ceratium hirundinella* | R | Wängberg 1995 |
| *Cosmarium tinctum* | R | Oliveira 1985 |
| *Cosmarium* sp. | R | Havas *et al*. 1995 |
| *Crucigenia tetrapedia* | S | Monteiro *et al*. 1995 |
| | S | Oliveira 1985 |
| *Cryptomonas marsonii* | S | Gächter and Máreš 1979 |
| *Cryptomonas ovata* | S | Gächter and Máreš 1979 |
| *Cyclotella bodanica* | R | Austin and Munteanu 1984 |
| *Cyclotella* spp. | S | Gächter and Máreš 1979 |
| *Desmids* | S | Kalin *et al*. 1989 |

| | | |
|---|---|---|
| *Dinobryon bavaricum* | S | Reinke and De Noyelles 1985 |
| | R | Havas *et al*. 1995 |
| *Dinobryon elegantissimum* | R | Oliveira 1985 |
| *Dinobryon sertularia* | S | Reinke and De Noyelles 1985 |
| *Elakatothrix* sp. | R | Reinke and De Noyelles 1985 |
| | S | Gächter and Máreš 1979 |

**Table 2.1** (continued)

| Species | Tolerance to metals | Reference |
|---|---|---|
| *Euglena mutabilis* | R | Kalin *et al*. 1989 |
| *Eunotia arcus* | R | Oliveira 1985 |
| *Fragilaria crotonensis* | S | Gächter and Máreš 1979 |
| *Fragilaria* spp. | S | Gächter and Máreš 1979 |
| *Gomphonema parvulum* | R | Loez *et al*. 1995 |
| | R | Monteiro *et al*. 1995 |
| | I | Oliveira 1985 |
| *Melosira distans* | S | Oliveira 1985 |
| *Navicula cryptocephala* | R | Austin and Munteanu 1984 |
| *Navicula* spp. | I | Oliveira 1985 |
| *Nitzschia frustulum* | R | Monteiro *et al*. 1995 |
| *Nitzschia palea* | R | Loez *et al*. 1995 |
| *Oocystis parva* | R | Moore *et al*. 1979 |
| *Ooocystis lacustris* | S | Gächter and Máreš 1979 |
| *Peridinium incospicuum* | R | Yan *et al*. 1985 |
| | R | Oliveira 1985 |

| | | |
|---|---|---|
| *Peridinium cinctum* | R | Wängberg 1995 |
| *Phacotus* | S | Gächter and Máreš 1979 |
| *Pseudoanaboena catenata* | R | Oliveira 1985 |
| *Quadrigula closterioides* | S | Oliveira 1985 |
| *Rhabdoderma gorskii* | R | Reinke and De Noyelles 1985 |
| *Rhaphidium* | R | Gächter and Máreš 1979 |
| *Rhizosolenia eriensis* | R | Deniseger *et al*. 1990 |
| | R | Havas *et al*. 1995 |
| *Rhodomonas lacustris* | S | Gächter and Máreš 1979 |
| *Rhodomonas minuta* | S | Monteiro *et al*. 1995 |
| *Scenedesmus armatus* | R | Monteiro *et al*. 1995 |
| *Scenedesmus intermedius* | R | Oliveira 1985 |
| *Scenedesmus quadricauda* | R | Oliveira 1985 |

**Table 2.1** (continued)

| Species | Tolerance to metals | Reference |
|---|---|---|
| *Schroederia setigera* | R | Gächter and Máreš 1979 |
| *Staurastrum* | S | Gächter and Máreš 1979 |
| *Stephanodiscus hantzschii* | S | Monteiro *et al*. 1995 |
| *Synedra acus* | R | Austin and Munteanu 1984 |
| | R | Havas *et al*. 1995 |
| *Synedra filiformis* | R | Austin and Munteanu 1984 |

| | | |
|---|---|---|
| *Synedra ulna* | S | Monteiro *et al*. 1995 |
| *Synura petersenii* | R | Oliveira 1985 |
| *Tabellaria fenestrata* | S | Austin and Munteanu 1984 |
| *Tabellaria flocculosa* | S | Austin and Munteanu 1984 |
| | S | Oliveira 1985 |
| *Trachelomonas volvocina* | R | Kalin *et al*. 1989 |

polluted Lago d'Orta (Bonacina *et al*. 1988). However, in Buttle Lake (Austin and Munteanu 1984), chlorophytes were more sensitive than diatoms. The response of diatoms is indeed the most inconsistent, going from resistance in the above-mentioned Buttle Lake and in Clearwater Lake (Yan 1979) to sensitivity in the Portugal reservoirs (Oliveira 1985) and in Lago d'Orta (Bonacina *et al*. 1988).

2.3.3.3 Canonical analysis of the entire community

Entire community inference models, based on canonical correspondence analysis, have been applied very successfully to examine the effect of acidification and eutrophication on diatoms (Hall and Smol 1992). This approach has been applied to the lakes around Sudbury (Dixit *et al*. 1989, 1991; Nicholls *et al*. 1992) as discussed in section 2.2.1.2. In these studies in which lakes were contaminated both by metal and acid, it proved somewhat difficult to separate the effects of the two stresses. This approach merits further validation especially in a series of lakes polluted mainly by metals. When sedimentary chrysophytes and diatoms are used, this method can be applied to monitor past episodes of pollution or to reconstruct the composition of pre-pollution assemblages on which to judge the recovery of the system. This method demands a highly trained technician able to accurately classify the algae. The development of a calibration set using a large number of sites along a gradient of metal pollution is essential to this method. This calibration requires a strong effort but the cosmopolitan distribution of many algae may allow its application to larger geographical areas.

### 2.3.3.4. Size distribution

Because taxonomic analyses require highly trained specialists, community size structure has been proposed as an alternative indicator of environmental perturbation (Sprules and Munawar 1986; Cattaneo *et al.* 1995). Laboratory and microcosm experiments with metals and other contaminants have generally induced a decrease in average phytoplankton size (O'Connors *et al*. 1978; Menzel 1980; Sanders and Cibik 1988; Munawar and Legner 1993). Enumeration and cell measurements necessary for size analyses can be done with a microscope and a computer-aided image analyzing system. Electronic particle counters can also be used to speed the size analysis. The best method is to use flow cytometry that allows fast counting and selection of cell types (Yentsch *et al*. 1983). Unfortunately this technique is limited by the expense of the equipment. This technique has been recently applied for monitoring effects of acidification consequent to open-pit lignite mining in five Bavarian lakes (Germany). Changes in pigment diversity and biomass spectra were observed in the most severely impacted lakes (Steinberg *et al*. in press). This technique provides fast and promising results, but unfortunately the expense of the equipment presently limits its utilization.

## 2.3.4 Tolerance

In several of the examples, authors have suggested that species have developed a tolerance to metals. This has also been confirmed in laboratory experiments where algal strains isolated from contaminated sites were more tolerant to metals (Stokes *et al*. 1973; Foster 1977; Takamura *et al*. 1989). As discussed in previous sections, under the impact of metals, community tolerance would increase by shifts to more tolerant species or, within a species, to more tolerant strains. While this development of tolerance confuses the interpretation of biomass and species composition responses to metal pollution, it can be advantageously used to test if a community has been exposed to metal pollution by simple short-term physiological test. This method, called Pollution Induced Community Tolerance (PICT) was first introduced by Blanck *et al.* (1988) and applied successfully by Wängeberg (1995) to a series of Swedish lakes differently affected by a smelter. For this method, it is necessary to establish a base-line tolerance for reference communities known not to have been exposed to the toxicant. The tolerances of the exposed communities are then compared with the baseline tolerance, and increased tolerance is a sign of the community having been previously affected by the toxicant. The interpretation of these results could be complicated by the possibility that exposure to one stress could induce tolerance to another. This phenomenon has been demonstrated for animals: prior exposure of the mussel *Mytilis edulis* to Cd, Cu, and Zn induced subsequent tolerance to Hg (Roesijadi and Fellingham 1987).

### 2.3.5 Biochemical and physiological indicators

#### 2.3.5.1 Pigments

In cultures, metals reduce chlorophyll, the chlorophyll a/b ratio, and pheophytin levels, but usually increase the carotenoid/chlorophyll ratio (Rai *et al*. 1981; De Filippis and Pallaghy 1994). The simplest explanation consistent with these observations is that metals inhibit the reductive steps in the biosynthetic pathway of photosynthetic pigments. Some of these trends have been confirmed with natural planktonic populations. When phytoplankton of the river Ganga was incubated with Cr and Hg, the ratio of carotenoids/chlorophyll, measured as the ratio of the absorbances of acetone extract at 430 and 665 nm (Singh and Rai 1991), increased. The hypothesis that metals induce a decrease in the ratio of chlorophyll a/phaeophytin has been so far confirmed only for mosses from sites contaminated either by metals or organics (Lopez and Carballeira 1989). Pigment analysis by High Performance Liquid Chromatography (HPLC) is becoming more and more available and allows quantification not only of the different chlorophylls, but also of their degradation products and of accessory pigments like luteins, fucoxanthine etc., typical of different algal classes. This method should provide a finer tool to monitor changes induced by metals in the pigments composition of algal populations and, at the community level, shifts in taxonomic composition. This approach has been used successfully in the estuary of the river Elbe (Germany) to distinguish between water masses characterized by different algal groups and differentially associated with the transport of particulate metals (Wiltshire *et al*. 1995).

#### 2.3.5.2 Phytochelatins

Phytochelatins are metal-binding peptides produced enzymatically by higher plants, fungi and algae in response to exposure to metals. These compounds detoxify intracellular metals by binding them through a thiolate coordination. They are equivalent in function but not in chemical composition to the metallothioneins found in animals and procaryotic organisms. Not all metals are equally effective in inducing phytochelatins in algae. In cultures of the marine diatom *Thalassiosira weissflogii* exposed to a series of metals (Cd, Pb, Ni, Cu, Zn, Co, Ag, and Hg) at concentrations similar to those in the marine environment, Cd and to a lesser extent Cu and Zn were the most effective inducers of phytochelatins (Ahner and Morel 1995). So far, phytochelatins have been studied only in marine species but they are widespread since they have been measured in all the species tested at metal concentrations encountered in natural waters (Price and Morel 1994; Ahner *et al*. 1995). Besides in culture, phytochelatins have also been measured in natural phytoplankton in Massachusetts Bay; a decreasing trend in phytochelatins was observed when sampling from the Inner Boston Harbour, which receives sewage and riverine inputs, to sites in the Bay farther from anthropogenic sources of metals (Ahner *et al*. 1994). Phytochelatins can be measured relatively easily by HPLC chromatography (Ahner *et al*. 1995). This technique is promising as an early warning of metal contamination since phytochelatin production is induced at metal concentrations too low to affect algal growth.

#### 2.3.5.3 Alkaline phosphatase

Alkaline phosphatase activity (APHA) catalyzes the hydrolysis of dissolved organic phosphate by phytoplankton and is usually inversely correlated to the phosphorus nutritional status of the algae. This enzyme is inhibited by copper in phytoplankton cultures (Rueter 1983). If confirmed in natural populations, this inhibition would have ecological implications; metals might affect the phosphorus nutrition of the algae at concentrations that would not be acutely toxic to the whole organism. This depression of alkaline phosphatase activity by metals could be used as a biochemical marker for overall toxicity. Measurement of APHA is straightforward and demands only a spectrophotometer or a fluorometer. However much more testing would be needed to confirm the utility of this test for biomonitoring purposes.

### 2.3.6 Morphological indicators

#### 2.3.6.1. Diatom deformities

Acute metal contamination has often been associated with the appearance of diatom deformation. Asymmetric frustules of *Synedra* have been observed in Lago d'Orta, a Northern Italian lake industrially polluted with copper (Tonolli 1961). Similar deformations were described for *Fragilaria* growing in the Eagle River, in the mining region of Colorado's southern Rocky mountains. Percent abnormal valves was significantly correlated with the concentrations of dissolved Cd, Cu, Fe, and Zn (McFarland and Hill 1997). In cores from Hamilton Harbour (Lake Ontario, Canada), Yang and Duthie (1993) observed abnormal *Stephanodiscus* frustules. The occurrence and increased abundance of these teratological forms coincided with records of metal pollution in the harbour. In laboratory cultures, morphological abnormalities were induced by elevated concentrations of Cd, or Zn and Cu both in freshwater (Adshead-Simonsen *et al*. 1981; Fisher and Jones 1981) and marine diatoms (Canterford 1980). These morphological alterations could be an alternative indicator of metal-induced stress. Percentages of deformed frustules are easily measured with a microscope and do not require highly trained personnel. Because diatom silica frustules are conserved in the sediments, deformities could be used also for monitoring past episodes of metal pollution. However, we do not know yet how widespread the occurrence of these abnormalities is in metal contaminated environments. Because diatom teratologies can be induced by silica deficiency, salinity, and extreme pH (Barber and Carter 1981), they cannot be indiscriminately ascribed to elevated metal concentrations.

#### 2.3.6.2 Algal size

In algal cultures exposed to high levels of metals, uncoupling of photosynthesis from cell division may result in increased cell volume (Stokes *et al*. 1973; Davies 1976; Christensen *et al*. 1979; Fisher and Jones 1981). These giant cells have been observed in different algal classes and in response to increased levels of Cu, Mn, Hg and Pb. Probably this is a short-term effect since these cells would not be viable and would be replaced by more tolerant species in cases of chronic pollution. In cultures where cell division was not inhibited, metal addition has instead resulted in size reduction (Shabana *et al*. 1986; Gensemer 1990). Response of algal populations to chronic pollution can be gleaned by paleoecology. In a core from Lago d'Orta, length of *Achnanhes minutissima* was significantly decreased in the sections coinciding with the metal pollution (Cattaneo *et al*. submitted). Clearly more studies of natural populations in chronically polluted environment are necessary before an index based on algal size can be recommended.

## 2.4 CONCLUSION

Phytoplankton responds to metal contamination with changes in species composition, size, morphology, and biochemistry. These changes can be used for monitoring the effects of mine activities on freshwater communities. Some of the techniques proposed here circumvent the need for taxonomical identification and therefore do not require technical specialists, but often do require sophisticated instruments. On the other hand, taxonomic analyses, after the initial investment in training, require inexpensive materials, minimal equipment, and the samples can be stored almost indefinitely.

## 2.5 RESEARCH NEEDS

Many interesting approaches are emerging as potential monitoring tools. However, in practical application, only traditional methods based on biomass changes and species shifts have been used so far. This review has identified 6 approaches that promise to be more powerful than the traditional ones and that could be integrated in a monitoring program with relatively low effort and cost: 1) Canonical analysis based on metals, 2) Effects of metals on community size distribution, 3) Pigment analysis using the entire absorbance spectrum or HPLC chromatography, 4) Detection of phytochelatins in freshwater algae, 5) Diatom deformities for monitoring present and past pollution, 6) Tests based on community induced tolerance.

Before these methods can be routinely used, however, it will be necessary to do a field validation at the mine sites. Some of the methods (e.g. canonical analysis) have been used for other forms of contamination and must be calibrated for metal pollution. Some are based on mostly anecdotal observations (size changes, pigment composition, deformities) often restricted to a region (tolerance tests) and their generality should be tested. For all methods, experimentation is needed to establish their detection limits.

# 3. PERIPHYTON

by

Raynald Chassé

## 3.1 OVERVIEW OF BIOMONITORING USING PERIPHYTON

Introduced in 1928 by Behning, Aperiphyton@ is defined as the heterogeneous and complex micro-community developing on natural and artificial submerged substrates (Weitzel 1979; Wetzel 1983). Substrates may be organic or inorganic, or living or dead material. Periphyton includes bacteria, yeast, mushrooms, protozoa, micro-algae and small invertebrates. Some authors associate this broad definition with the term A*aufwuch*@, and reserve the term periphyton for the micro-algal communities. Attached Benthic Micro-algae (ABM) is also a term used by some authors. A distinction is made between the epilithon, that is the periphyton on rock substrates, epipelon on mud or silt substrates, epipsammon on sand substrates and epiphyton on the submersed portions of aquatic macrophytes (Aloi 1990). In this document, periphyton corresponds to the term defined originally by Behning.

Periphyton, more particularly the micro-algal component, is both an important primary producer in rivers or in littoral zones of lakes (Cattaneo and Kalff 1980) and a food source for invertebrates (Soska 1975). This community represents a functional system where autotrophic and heterotrophic processes are taking place concurrently. Periphyton is the interface between the substrata and the surrounding waters. Consequently, periphyton influences the biogeochemistry and the dynamics of ecosystems (Wetzel 1983). Because of its sedentary nature, periphyton is a good indicator of local conditions and the micro-algal fraction plays a potentially important role in the trophic transfer of contaminants.

For all these reasons, many studies have used periphyton for assessing water quality (Chessman 1985; Clements and Kiffney 1994; Cattaneo *et al.* 1995). It integrates effects of environmental variables (Marcus 1980; Round 1991), has short response times to disturbances and persists even under severe perturbations.

## 3.2 CASE STUDIES

### 3.2.1 Periphyton and biomonitoring at mine sites

#### 3.2.1.1 Buttle Lake, Vancouver Island, British Columbia

One of the major research efforts in Canada on the impacts of mining activities on periphytic communities is certainly the investigation of Austin and his team in central Vancouver Island, British Columbia (Brown and Austin 1971; Austin *et al.* 1981; Nordin *et al.* 1985; Deniseger *et al.* 1986). During the late 1960's, prior to the disposal of mine tailings in Buttle Lake, an investigation was undertaken to accumulate baseline data on periphyton in the relatively undisturbed and oligotrophic lake (Brown and Austin 1971; Brown 1973). Subsequent studies in the 1980's sought to evaluate changes in the periphyton communities following a fourteen year exposure to tailings effluent (Austin 1983; Lang and Austin 1984; Austin and Deniseger 1985; Austin *et al.* 1985; Roch *et al.* 1985; Lucey *et al.* 1986). During these studies, a number of alternative field methods were compared with other commonly employed methods.

Prior to the 1967 discharge of tailings containing metals (Cd, Cu, Pb and Zn) into Buttle Lake, Clark (1980), Clark and Morrison (1982) and Austin (1983) demonstrated that the periphytic micro-algal community composition and density were not significantly different among the sampled sites. These communities were dominated by species indicative of well oxygenated, clean, oligotrophic waters. After the onset of tailings discharge, community composition was significantly altered, with shifts in dominant associations and losses of both previously abundant and rare forms known to be intolerant of metal stress. Other more tolerant forms, previously rare, increased in abundance (Austin 1983). Austin and Deniseger (1985) found, after a seventeen-year study of the community composition of Buttle Lake, significant correlations between temporal and spatial variations in periphyton composition and the horizontal metal gradient within the lake.

At the same time, a study was conducted in a small creek - Myra Creek- a tributary of Buttle Lake (Deniseger *et al.* 1986). Two stations were chosen for this study, one above the mine and one located downstream near Buttle Lake, on the basis of similarity of flow, depth, light, temperature and mixing conditions. Glass microscope slides were incubated for ten weeks for periphyton accumulation. Comparative studies of periphyton were enhanced by measures of similarity between samples. The indices used were the dissimilarity index (Levandowsky 1972), community diversity (Shannon 1948) and species evenness (Pielou 1977). The authors concluded that natural factors can play an important role in community structural responses to metal contamination. In fact, they observed significantly different reactions to metal contamination between spring and summer communities. They demonstrated that temperature and high flow rates played a key role in the seasonal sequences of periphyton communities. Species diversity and evenness were similar upstream and downstream of the effluent in the spring samples, whereas in summer these indices indicated large differences. Comparisons of community structure by the dissimilarity index, which takes into account the number of species and their relative distribution, also indicated a highest dissimilarity in the summer samples. This increase of dissimilarity was associated with the growth pattern of green algae. Within this class of algae, they observed positive correlations between increasing concentrations of various metals (Cd, Cu, Pb and Zn) and higher numbers of *Achnanthes minutissima*.

#### 3.2.1.2 Sudbury, Ontario

The Sudbury region has been studied extensively for effects on lakes contaminated by mining activities. However, few studies used periphyton for monitoring the impacts. In 1975, Hutchinson *et al.* used periphyton to monitor bioaccumulation of Cu, Ni and Zn released from mining activities in the Sudbury region. They concluded that when several species of rooted macrophytes, zooplankton, fish, crayfish, clams and periphyton were sampled in contaminated areas, metal concentrations in periphyton were among the highest measured.

Some authors also studied the impact of pH on periphytic communities of many lakes in the Sudbury region (Stokes 1986; Turner *et al.* 1987). Jackson *et al.* (1990) and

Vandermeulen *et al*. (1993) studied the effects of pH changes in several soft-water lakes on the composition of filamentous green algae communities. They observed that the dominant factor correlating with the abundance of some particular species (among others *Zygogonum tunetanum*) appeared to be the pH. However, they also observed, in addition to pH effects, changes in the concentration of major ionic constituents and metals. These authors proposed that the dominance of particular filamentous green algae probably resulted from complex interplay between the direct physiological effect of pH variations and secondary effects due to pH variations on the lake ecosystem.

#### 3.2.1.3 The Northwest Miramichi River system, New Brunswick

Besch *et al*. (1972) studied the effects of acid and metal mining contamination on benthic diatom communities collected from polyethylene and natural substrata in a river system free of any other kind of pollution. The aim of their study was to find an indicator system based on periphyton which would allow biomonitoring of the Northwest Miramichi River and its tributaries (Tomogonops River, South Tomonogops River and Little South Tomonogops River), contaminated principally by Cu and Zn due to mining activities and tailings. Contaminated and reference areas (Sevogle River) were characterised by low pH, particularly at stations immediately below mines (as low as pH 1.9). Artificial substrata were distributed at thirteen stations and exposed from April 1968 to July 1969, except during winter months when samples were taken from stones at the same stations. Identifications were conducted and relative abundances were estimated for the diatom community.

Results indicated that the pH is indeed the most decisive factor in regulating the occurrence of the most abundant species. Diatoms showed seasonal patterns in abundance but within a given season, highest abundances of the most numerous species were reached at their optimal pH. Therefore, researchers concluded that diatoms can be considered as reliable indicators of the pH and its fluctuation. However, results also indicated that the task of indicating metal contamination and its magnitude are more complex. In the study, the presence of metals was not reflected by indicator species but by the dominance of tolerant species, with a simultaneous absence of all less tolerant forms. From these observations, Besch *et al.* (1972) proposed an indicator system based on tolerance of some common species in the Northwest Miramichi area for Zn and their pH preference range. However, according to these authors, this preliminary indicator system comprises only species occurring in appreciable abundances in the study area and is applicable only for streams of the soft water zone of the Atlantic Provinces of Canada, which do not received organic contamination.

#### 3.2.1.4 Other mine sites

*Prickly Pear Creek, Montana, U.S.A.*

Crossey and La Point (1988) studied periphyton community function and structure simultaneously, in order to compare them as indicators of environmental impact due to metal contamination in Prickly Pear Creek, Montana. Their study area was a river draining the Colorado Mining District that was contaminated with high concentrations of Cd, Cu, Pb, Ag and Zn which were leached from waste piles associated with milling and smelting operations. Three study sites (control, impacted and recovered) were intensively studied and chosen to minimize differences in abiotic factors shown to be important in periphyton colonization studies. Artificial substrates (granite slabs) were used. Production and respiration measurements were conducted over a period of ten days and structural parameters were chlorophyll-a, ash-free dry mass (AFDM) and taxonomic analysis (limited to diatoms).

Results indicated that changes had occurred in the periphyton community. The authors were able to distinguish statistical differences among control, impacted and recovery reaches using some parameters, both structural and functional. However, the researchers demonstrated that primary production and photosynthetic efficiency could not be differentiated among stations, even though mean values appeared fairly different. On the other hand, AFDM and chlorophyll-a showed a significant increase from the control to the impacted reaches. This study demonstrated that structural parameters allowed one to detect significant changes within the impacted zone. There was a decrease in community complexity with declining species diversity and richness and increasing cell abundances. This result points to a loss of sensitive species with a concomitant proliferation of more resistant species.

*Arkansas River, Colorado, U.S.A.*

Clements and Kiffney (1994) employed an integrated laboratory and field approach to assess the effects of metals at a Superfund site of mining contamination on the Arkansas River. Ambient metal levels, chronic toxicity tests with the micro-crustacean *Ceriodaphnia dubia*, metal accumulation by periphyton and benthic invertebrates, and benthic invertebrate community structure were examined at stations located upstream and downstream from the site contaminated by Cu, Cd and Zn. Field and laboratory data (from whole rocks for periphyton) were collected in fall 1990 and during 1991.

Results indicated that levels of Cd, Cu and Zn in periphyton and benthic invertebrates were significantly more elevated at stations downstream from the source of contamination during both seasons. Considerable variation among organisms was observed. Metal levels were generally much higher in periphyton than in the benthic invertebrates. Seasonal variation in metal levels in periphyton was observed, but this variation was not always related to ambient concentrations. Whereas Cd levels in periphyton were elevated in the spring, levels of Cu and Zn in periphyton were higher in the fall, despite lower ambient levels. Researchers concluded that their results supported the findings of other researchers and demonstrated that the level of metals in periphyton is a good indicator of potential impacts and that total metal concentrations in water were not good predictors of bioavailability.

*Miyata River, Japan*

Seasonal changes in species composition and photosynthesis of the periphytic community were investigated in the Miyata River contaminated by Cu mining (Takamura *et al.* 1990). Mine activities began in 1908 and closed in 1981; known contaminants include Cu, Zn, As, Cd and Pb. The mean concentrations of Cu and Zn in the river during the study were 100 and 400 μg/L, and for Cd, Pb and As respectively, mean concentrations were 5, 50 and 20 μg/L. This study was carried out monthly at three sites on the river from February 1987 to January 1988. Samples of periphyton were collected from several cobbles for identification, enumeration, algal biomass determination and photosynthesis measurements.

Results demonstrated that the periphyton standing crop, measured as chlorophyll-*a*, varied widely even at the same time and site. However, contrary to the observations of Leland and Carter (1985), the biomass of periphyton in the Miyata River did not decrease in the presence of Cu. Takamura *et al.* (1990) concluded that this high biomass of periphyton in the river may be due to extremely low grazing pressure because of the poorly developed aquatic insect community. On the other hand, researchers observed that the number of species was restricted to only 7 to 10. Bacillariophycae, such as *Achnanthes minutissima*, were co-dominant throughout the year. This species is also reported to be dominant in Cu-contaminated rivers (Deniseger *et al.* 1986; Leland and Carter 1984). These authors also observed that despite the high nutrient concentrations, the photosynthetic rates were lower than those for uncontaminated rivers in the literature. Their studies suggest that this limited algal photosynthesis is the result of the complex contamination of the Miyata River. However, the authors did not indicate whether abiotic factors such as light conditions and current velocity had been taken into account.

### 3.2.2 Periphyton in non-mine-related biomonitoring studies

#### 3.2.2.1 Convict Creek, California, U.S.A.

Leland and Carter (1984, 1985) studied the effects of Cu on the periphytic communities of oligotrophic Convict Creek, in a reserve of the Sierra Nevada Aquatic Research Laboratory. Convict Creek is a perennial riffle-pool stream with more than 80% riffles in the study area. The main channel is divided into two parallel 0.8 km sections, within four stream sections ranging in length from 340 to 500 m in which discharge can be regulated. Experimental sections were dosed continuously with cupric sulphate from mid-August 1979 to mid-January 1979. Periphyton were collected from cobbles and artificial substrates (glass slides) colonized for 3 weeks before collection. Changes in species composition, biomass, phytopigment, adenosine triphosphate, ash-free dry weight, primary production, nitrogen fixation and processing of leaf litter were examined in experimental sections, continuously dosed for 1 year at three nominal concentrations of Cu (2.5, 5 and 10 μg/L $Cu_t$).

Results demonstrated, in accordance with previous research, that the numerically most abundant taxa were Bacillariophyceae *(Achnanthes minutissima, Cocconeis placentula, Cymbella microcephala, Fragilaria* spp. etc.). Researchers also observed the cyanophyte *Lynglya* spp., a codominant species during spring and summer. Population densities of *Lynglya* spp. were markely reduced at all test concentrations of Cu. Population densities of the principal Chlorophyta (*Spirogyra* spp. and *Cladophora* spp.) and the diatom *Amphipleura pellicida* were reduced at 5 μg/L $Cu_t$. *A. minutissima*, a co-dominant in the control, was the primary replacement species. Other taxa that were more abundant at 5 μg/L $Cu_t$ than in the control were *Ceratoneis arcus*, *Cocconeis placentula*, *Navicula* spp. and *Synedra rumpens*. Only *A. minutissima* and *Calothrix* spp. were more abundant at 10 μg/L $Cu_t$ than in the control. Of the 22 most abundant taxa, 16 were reduced in abundance by continuous exposure to 10 μg/L $Cu_t$. From these results, Leland and Carter compared three resemblance measures (Canberra metric, Bray-Curtis and Dice) and diversity (Brillouin:s) for detecting differences in species composition among experimental stream sections. They concluded that the Canberra metric, an index sensitive to proportional rather than absolute differences, was the most informative of these indices.

In addition to stuctural parameters, the authors also compared several functional parameters. They observed that autotrophic productivity was reduced by 51-81% at 2.5 μg/L $Cu_t$, 55-96% at 5 μg/L $Cu_t$ and 81-100% at 10 μg/L $Cu_t$. Heterotrophic productivity (based on dark $^{35}$S-sulphate uptake) was inhibited to a lesser extent (28-63% at 2.5 μg/L $Cu_t$, 24-84% at 5 μg/L $Cu_t$ and 67-92% at 10 μg/L $Cu_t$). The inhibition of autotrophic and heterotrophic productivity persisted through the year of exposure. Production in stream sections previously exposed to 2.5 and 5 μg/L $Cu_t$ increased to control levels within 4 weeks after dosing was ceased, but remained depressed for more than 7 weeks after exposure to 10 μg/L $Cu_t$. The specific rate of photosynthesis (mg C mg /chlorophyll a/h) of mature periphyton communities declined at all test concentrations of Cu, but the rate for periphyton on newly-colonized surfaces did not change. Nitrogenase activity was inhibited during the initial weeks of exposure by 5 and 10 μg/L $Cu_t$, but, after 9 months of exposure, control and Cu-treated sections did not differ. Finally, the rate of processing of leaf litter, estimated by microbial respiration and nutrient quality of litter of resident riparian woodland taxa, was inhibited at all test concentrations of Cu, but there was no commensurate reduction in standing crops (total number of individuals of all taxa).

The researchers concluded that their results reflected a change in composition of periphytic communities during the year of exposure to an assemblage tolerant of Cu. Also, although the species composition and structure of diatom assemblages seemed to indicate environmental deterioration, structure and function of aquatic communities are coupled in a complex manner not easily quantified. Consequently, taxonomic descriptions may not detect significant changes in process rates affecting the community.

#### 3.2.2.2 Calcasieu River System, Louisiana, U.S.A.

Ramelow *et al.* (1987) determined the levels of Cu, Pb, Cr, Zn, Cd, As and Ag in periphyton specimens obtained with a diatometer collector along three important bayous of the Calcasieu River System. This system is an area where heavy industry and urban discharges are believed to have severely altered water quality; several stations were selected taking into account two principal types of contamination (petrochemical industrial discharges and urban runoff) and including an uncontaminated control.

Results indicated that the periphyton tended to concentrate As, Cd, Cr and Zn far above levels measured in water and sediments collected at the same stations. However, the metals Pb and Cu were not enriched in the periphyton, except near known sources of metals. The researchers concluded that this fact was probably due to the high concentrations of these latter two metals in the water column near contaminated stations. Thus, concentration ratios of periphyton over sediment greatly exceeded one for the metals Cr, Zn, Cd, As and Ag. From their results, the authors concluded that the concentrations of metals in the periphyton appeared to yield more information about contaminants than either water or sediment samples. However, Ramelow *et al.* (1987) indicated that the cleaning of periphyton samples for observation had limited their analysis only to diatom taxa. This problem was reported by other researchers (Newman *et al.* 1983, 1985).

#### 3.2.2.3 Uintah Basin, Utah, U.S.A.

Rushforth *et al.* (1981) studied the relationships between attached diatom species and dissolved metals in the streams of the Uintah Basin of Utah through four seasons of 1977 to 1978. Niche center gradient analysis (index calculated to indicate the relationship of diatom species to an environmental gradient), cluster analysis and correlation analysis were performed. The Uintah Basin is a large, natural depression lying in northeastern Utah and Colorado. Attached algae from natural substrates were analyzed along with aqueous contentrations of 14 metals.

The results demonstrated that under field conditions several different diatom species showed different patterns of abundance with varying concentrations of metals. Thus, *Achnanthes minutissima*, *Cymbella minuta*, *Gomphonema parvulum, Navicula secreta*, *Synedra ulna* and some species of *Nitzschia* appeared to be indicator species of low metal concentrations, while *Achnanthes microcephala*, *Eunotia exigua*, *Pinnularia interrupta* and some species of *Fragillaria* appeared to be very resistant. However, although different analyses used for determining correlations between biological association patterns and metal concentrations have demonstrated their value, these researchers have also shown that such responses may relate to seasonal differences.

## 3.3 CRITICAL EVALUATION OF THESE AND OTHER POTENTIAL MONITORING METHODS

This section reports on four main approaches that have been used to monitor water quality with periphyton and analyzes general problems met with field methods.

### 3.3.1 Indicator species

The first main approach used to monitor water quality using periphyton consists of using the presence and abundance of indicator species (Patrick 1949, 1978; Cholnoky 1958; Patrick and Reimer 1966; Lowe 1974; Lange-Bertalot 1979). The first studies establishing the ASaproby system@ described by Kolkwitz and Marsson (1967) go back to the end of last century (Sladecek 1973). However, this notion of indicator species seems very subjective (Wetzel 1983). In fact, studies based on species associations are more powerful than those taking only individual indicator species into account (Sladececk 1973; Collins and Cornelius 1978).

The most frequent use of periphyton to assess the effects of altered water quality is the identification of community or species shifts. Some researchers discuss alterations in broad taxonomic groups, while others focus on particular sensitive species. Some field studies, such as those conducted in lakes receiving metals from mine drainage (Austin and Deniseger 1985; Austin *et al*. 1985; Roch *et al*. 1985), have identified shifts in species composition with loss of metal-sensitive species.

Diatoms represent, in general, an essential portion of periphyton and are considered good indicators of water quality (Patrick 1971, 1973; Collins and Cornelius 1978; Coste 1978; Weitzel 1979; Lafont *et al.* 1988; Round 1991). In fact, the species composition and structure of diatom assemblages are frequently emphasized because species are easily identified in preserved samples and because such information can indicate environmental deterioration (Weber 1973; Patrick 1976; Weitzel 1979). Weber and McFarland (1981) observed significant changes in the species composition of the periphyton of a eutrophic calcareous stream continuously dosed with 120 μg/L of Cu. Two numerically dominant algal species were eliminated by Cu but their replacement by 3 non-dominant species and the increase in abundance of several other species produced a greater diversity and comparable standing crops in the contaminated reaches than in the control. Genter *et al.* (1987) observed that different concentrations of Zn treatment could be identified by a particular periphytic alga taxa depending on the season studied. In their study, they observed that the 0.05 mg Zn/L treatment could be identified by certain diatom taxa being more abundant than in a control in spring and by a filamentous green alga in summer and fall. On the other hand, for the 0.5 mg Zn/L treatment, filamentous green alga were most abundant only in fall; at 1.0 mg Zn/L treatment the periphytic community was dominated by unicellular green algae in all seasons except in summer where these species were co-dominant with filamentous blue-green algae. The authors concluded that Zn bound to periphyton was much better for identifying Zn contamination than total Zn in water. Similarly, Rushforth *et al.* (1981) demonstrated that under field conditions several diatom species show different patterns of abundance with varying concentrations of metals. Genter *et al.* (1987) have shown that such responses may be related to seasonal differences.

Although metal contamination clearly determined which taxa would dominate, cause-and-effect relationships between metal presence and species responses are often difficult to detect. This is due to the fact that environmental factors do not operate separately from one other, but rather in synergistic or antagonistic ways (Evenson *et al.* 1981). In fact, the development of periphyton is affected by many natural factors. Among these, light, nutrients, water movements, nature of the substrata and grazing are the most important (Weitzel 1979; Roos 1983). All these factors directly influence periphytic community responses to environmental stress. There is no doubt that light is very important for periphyton development. Some species such as some cyanobacteria (Weitzel 1979) or filamentous chlorophycea (Shortreed and Stockner 1983; Steinman and McIntire 1987) are favoured by high light intensity while others such as diatoms show a wide interspecies variation (Hudon and Bourget 1981; Antoine and Benson-Evans 1983; Steinman and McIntire 1986). Also, as for phytoplankton, periphyton growth reflect nutrient limitations (Stockner and Shortreed 1978; Marcus 1980; Peterson *et al.* 1983; Fairchild *et al.* 1985). In general, nitrogen and phosphorus enrichment translate into important increases in periphytic biomass (Marcus 1980; Perrin *et al.* 1987; Fairchild and Everett 1988). Similarly, species in rivers have a well defined niche corresponding to a specific current velocity (Traaen and Lindstrom 1983). In general, high velocities inhibit development of periphyton, except for filamentous species (Weitzel 1979). Finally, grazers may reduce biomass (Power and Matthews 1983; McAuliffe 1984; Jacoby 1987), primary productivity (Gregory 1980; Power 1984; Lamberti and Resh 1985) or may influence species composition (Hart 1981; Power *et al.* 1985; Lamberti *et al.* 1987; Steinman *et al.* 1987).

### 3.3.2 Mathematical index

The second main approach uses mathematical indices such as a biotic index, diversity and species richness (Patrick *et al.* 1954; Patrick and Strawbridge 1963; Cairns and Dickson 1971; Pielou 1977; Sullivan 1986; Lafont *et al.* 1988) or correspondence analysis such as niche center analysis, cluster analysis and correlation analysis (Rushforth *et al.* 1981; Niederlehner and Cairns 1992). Although the expected relationship between diversity and environmental stress has not always been confirmed in the field, generally mining effluents decrease richness and diversity (Austin 1983; Austin and Deniseger 1985; Crossey and La Point 1988; Patrick 1988) because of the disappearance of sensitive species (Patrick 1978; Weber 1981). On the other hand, correspondence analysis appears promising but requires more development.

### 3.3.3 Functional responses

The third main approach used to monitor water quality with periphyton consists of measuring functional responses (Eichenberger 1975; Wührmann and Eichenberger 1975; Collins and Cornelius 1978; Rodgers *et al.* 1979; Sand-Jensen 1983; Crossey and La Point 1988; Watanabe *et al.* 1988). According to Collins and Cornelius (1978) and Weber (1981), metabolic activities are the first to be affected by water quality alterations; community structure changes more slowly to adapt to the new field conditions. Thus, according to Watanabe *et al.* (1988), photosynthetic and heterotrophic activities would be more sensitive than indices based on species community. However, large natural variations in functional responses restrict their use as water quality indicators (Schindler *et al.* 1973). On the other hand, Rodgers *et al.* (1979, 1980) observed lower variability of functional parameters than structural parameters in experimental streams affected by Cu and Cr. These authors concluded that protection of the functional integrity of periphyton doesn't necessarily mean structural integrity protection. In fact, these two approaches are complementary.

Changes in tolerance of whole communities are difficult to predict from information on individual populations. While the mechanisms by which the tolerance of a population can change include physiological adaptation and genetic selection for resistance, the tolerance of a community of interacting organisms can change through additional mechanisms that are unique to communities. Thus, for example, Blanck and Wangberg (1988) found that previously exposed periphyton were better able to maintain their relative primary productivity in the face of subsequent arsenate exposure. Similarly, Niederlehner and Cairns (1992) found that periphytic communities developed under Zn stress were intially impaired but changed less in response to additional stress relative to their initial state. Thus, taxonomic composition changed less and gross primary productivity was less impaired by secondary stress in periphyton acclimated to Zn. These researchers also demonstrated that community respiration, algal biomass, total biomass and respiration to biomass ratios did not differ in response to secondary stress.

### 3.3.4 Bioaccumulation

Finally, the high concentration factors for metals noted for algae (Trolope and Evans 1976; Ramelow *et al.* 1987) and bacteria (Johnson *et al.* 1981) suggest a further potential to use periphyton to monitor metal contaminated sites (Ramelow *et al.* 1987; Newman *et al.* 1985). Like phytoplankton, this characteristic may be used to detect contamination. However, because of the complexity of the periphytic community, the interpretation of the relationships between algal metal accumulation and surrounding concentrations should be made with circumspection. In fact, the materials analysed to estimate accumulation were considered principally biotic, and abiotic factors involved have not always been taken into account. Newman and McIntosh (1989) suggested that the highest values of many metals reported by Hutchinson *et al.* (1975) in periphyton in regard to other organisms are an artifact of the analytical methodology. Indeed, the accumulation of metals by periphyton results from a suite of interrelated biogeochemical mechanisms. Everard and Denny (1985) concluded that increasing concentrations of Pb in periphyton samples was the result of periods of calm weather in the lentic system favoring flocculation of ecton. Similarly, Kinniburgh and Jackson (1981) explained accumulation in periphyton by coprecipitation of metals with hydrous oxides. Adsorption of metals on cell surfaces (Fujita and Hashizuma 1975) or on clays (Sakata 1987) and association with organic matter (Filipek *et al.* 1981) contribute also to accumulation of metals in periphyton. The periphyton itself can modify physico-chemical conditions in the micro-layers with photosynthesis and respiratory activities (Newman and McIntosh 1989). Consequently, these alterations of physico-chemical conditions in the abiotic components of periphyton may enhance precipitation or dissolution of hydrous iron and manganese oxides or adsorption/desorption of contaminants. According to Sode (1983), species number, biomass and productivity of periphyton can be significantly influenced by hydrous iron and manganese oxides. Finally, Newman *et al.* (1983, 1985) concluded that determination of the accumulation of metals by cells, principally in filamentous algae, can be an insurmountable problem for biomonitoring studies.

### 3.3.5 Field methods

The application of monitoring methods using periphyton to assess the impacts of mine effluents on the aquatic environment are limited by the need for field methods development and standardisation.

#### 3.3.5.1 Representative samples

A major difficulty in studying periphyton is obtaining representative and uniform samples, because of spatial heterogeneity and inherent problems with field sampling (Weitzel *et al.* 1979). Many methods of sampling have been suggested, from the simple, such as scraping or brushing (Hunding 1971; Moore 1974; Stanley 1976; Blinn *et al.* 1980; Ennis and Albright 1982; Power and Matthews 1983) to more complex ones such as manual aspiratory systems or portable peristaltic pumps (Eaton and Moss 1966; Stockner and Armstrong 1971; Hamala *et al.* 1981). However, because of differences of textures and surface irregularities of natural substrates, quantitative studies are difficult.

#### 3.3.5.2 Artificial substrates

To minimize these problems, many researchers have proposed to use artificial substrates; advantages and disadvantages have been discussed for a long time (Cooke 1956; Sladeckova 1962; Wetzel 1964; Patrick 1967; Weitzel *et al.* 1979; Austin *et al.* 1981). The principal advantages of artificial substrates are their ease of manipulation, their homogeneity, their uniform surface and the possibility of obtaining many replicates (Schindler *et al.* 1973; Lamberti and Resh 1985). These characteristics allow a reduction in the variability among replicates (Brown 1976; Weitzel *et al.* 1979). Furthermore, artificial substrates allow one to sample delicate species, usually destroyed by brushing or scraping natural substrates (Klasvik 1974) and generally there is a good similarity between species colonizing both types of substrates (Castenholz 1960; Peters *et al.* 1968; Klasvik 1974; Brown 1976; Hooper and Robinson 1976; Shamess and Robinson 1982 *in* Robinson 1983). However, some filamentous species such as *Cladophora*, *Oscillatoria*, *Phormidium*, *Spirogyra* and some red micro-algae colonize less on artificial substrates, particularly on substrates with smooth surfaces, than on natural substrates (Castenholz 1960; Klasvik 1974; Brown 1976).

#### 3.3.5.3 Experimental streams

Because periphyton development is affected by many natural factors, some researchers have used experimental streams to decrease the important variations in periphytic responses (McIntire *et al.* 1964; Warren and Davis 1971; McIntire 1975; Gerhart *et al.* 1977; Clark *et al.* 1980; Russel *et al.* 1981; Peterson *et al.* 1983; Reiter and Carlson 1986). These studies demonstrate the high potential of experimental streams for monitoring contaminants like metals (Goldsborough *et al.* 1986; Pontasch 1995).

The objective of all these systems is to control physico-chemical variables associated with periphyton development. The most controlled of these experimental streams allow one to isolate a few variables and facilitate the interpretation of observations, while the less controlled systems resemble the field (Clark *et al.* 1980). However, according to Pontasch (1995), more research is required to determine the acceptable gap between field conditions and controlled conditions of experimental streams.

## 3.4 CONCLUSION

The existence of many studies using periphyton as bioindicators may be explained by several factors. (1) The large number of investigations with periphyton in different systems facilitate interpretation. (2) The micro-algal fraction of periphyton plays an important role in the trophic transfer of contaminants. (3) Micro-algae exhibit high concentration factors for many metals (Newman and McIntosh 1989). (4) Bioconcentration data can be easily associated with results from community composition or processes. (5) When artificial substrates are used, they are inexpensive, easily fabricated and yield sufficient biological material during all seasons. (6) Utilisation of microcosms or enclosures permits an acceptable ecological realism when associated with monitoring in the field (Genter and Amyot 1994).

Although periphyton is being used increasingly to monitor metals in aquatic ecosystems, heterogeneity and complexity of the periphytic communities and the absence of methodological standardisation still limit its use in water quality impact studies. This fact explains why periphyton are less studied than phytoplankton (Rosemarin and Gelin 1978; Aloi 1990).

Controlled exposures to metals (Sigmon *et al*. 1977; Patrick 1978; Kaufman 1982) and biomonitoring studies of mining activities (Clark and Morrison 1982; Austin and Deniseger 1985; Austin *et al.* 1985; Nordin *et al.* 1985; Roch *et al.* 1985; Deniseger *et al.* 1986; Crossey and La Point 1988; Ramelow *et al.* 1992; Clements and Kiffney 1994; Vinyard 1996) suggest that periphyton biomass, species composition, successional processes and diversity may be effective indicators of metal impact during properly conducted field surveys (Newman and McIntosh 1989). While temporal comparisons are rarely possible, spatial comparisons are of considerable value (Stokes 1983).

Further, community processes such as primary productivity, autotrophic and heterotrophic production and nutrient cycling provide additional information with which to assess metal impacts on periphytic communities.

## 3.5 RESEARCH NEEDS

The major problem that confronts investigators using periphytic communities is interpreting the significance of changes observed following the presence of metals or other anthropogenic perturbations with regard to environmental factors.

As a matter of fact, the principal difficulty in determining the ecological significance of the effects of contamination due to mining activities on periphyton is to differentiate the natural variability of the measured variables from that induced by metal contamination. In all studies, where the objective is to estimate effects of metals on the periphytic communities, the choice of variables to measure is a critical step.

The absence of protocol standardisation also limits comparisons among studies on different systems and seriously handicaps studies on the function and structure of periphytic communities. In this context, more research should be carried out to define acceptable variability in parameters studied, principally for functional variables. Further, development of standardised protocols is essential to compare results between different studies.

Finally, critical areas requiring further research include assessment of the relative importance of biotic and abiotic components in determining metal concentrations in periphyton and examination of the bioavailability of metals associated with periphyton.

# 4. EVALUATION OF MONITORING METHODS USING MACROPHYTES, PHYTOPLANKTON AND PERIPHYTON

by

Christian G.J. Fraikin

## 4.1 INTRODUCTION

The overall objective of this technical evaluation was to review monitoring methods that make use of aquatic plants and to make recommendations as to the usefulness and cost-effectiveness of these methods. Each of the three main groups of plants (macrophytes, phytoplankton and periphyton) was evaluated separately by individual authors in the preceding chapters. The specific objective of this section was not to provide detailed descriptions of each method, as these are provided in the other chapters, but rather to critically evaluate the usefulness of these monitoring tools against a number of criteria, including:

- ecological relevance;
- validation;
- site specificity;
- applicability;
- repeatability;
- practical limitations;
- commercial availability;
- interpretability; and
- variability.

Not all criteria were addressed for each monitoring tool, because in many cases one or more criteria were already discussed under another criterion, a given criterion did not apply to the technique discussed, or it could not be evaluated due to lack of information in the scientific literature.

## 4.2 MACROPHYTES

Macrophytes are commonly used in the biomonitoring of community structures and in the measurement of metal contamination. Community measures in biomonitoring studies range from rough counts of species, presence/absence and abundance of certain indicator species and species diversity and richness analyses (Clements 1991).

### 4.2.1 Species and community composition

Streams and lakes exposed to high levels of metals can show differences in the standing crop, diversity and presence/absence of attached algal and rooted vascular plant communities when compared with similar but unexposed habitats (See Chapter 1). An assessment of the community structure in the exposed area not only provides direct evidence of impacts at the community level, but also provides clues as to the tolerance (species presence) or intolerance (species absence) of various species, which could then also be used as indicator species.

#### 4.2.1.1 Ecological relevance

Monitoring the structure of macrophyte communities can provide an indication of environmental impacts on aquatic ecosystems. Measures of species abundance, diversity and richness can reflect the general toxicity effects of potential contaminants in the system (Hellawell 1986).

#### 4.2.1.2 Validation

Many studies have demonstrated a reduction in species diversity and abundance in macrophytes exposed to metal-polluted waters (Gorham and Gordon 1963; Besch and Robert-Pichette 1970; Kelly 1988; Ripley *et al*. 1996). Gorham and Gordon (1963) observed a reduction in species diversity of submerged and floating macrophytes with decreasing distance from sulphur dioxide smelters in Sudbury, Ontario. Since the reduction of the emissions, ecosystem recovery was observed as an increase in species richness (Ripley *et al*. 1996). Similarly, Kelly (1988) compiled a list of species present along a contamination gradient near a smelter and found a gradual change in community composition: species numbers declined closer to the source and only a few tolerant species increased in density.

A more elaborate method of monitoring river pollution with macrophytes was developed by Haslam (1982). Her approach considers the difference in species diversity, vegetation cover, trophic status, pollution tolerance and in physical damage of macrophytes between reference and impacted areas. Small *et al*. (1996) proposed a similar method, expanding on this work to compare higher plant community associations with streams known to be of high quality and those from degraded systems.

#### 4.2.1.3 Site specificity

Given the overall variability of macrophyte community composition in aquatic systems, no specific species can be identified as an indicator. Rather, the community composition of the aquatic system investigated will determine the type of macrophyte selected.

#### 4.2.1.4 Applicability

Macrophytes possess many attributes that make them good biomonitors: they are visible to the naked eye, they are sedentary, they are easy to collect and to handle, and a sampling program requiring species identification and enumeration, is much less costly than field and laboratory exposure assessments.

#### 4.2.1.5 Interpretability

Evidence from several studies suggests that differences (between an exposed and unexposed site) within species may occur as either environmental or genetic responses to metal enrichment. Such adaptation by a species could mask the effects of the contaminant in question; therefore, knowledge of the potential adaptive characteristics of local species would be required to properly interpret surveys of community structure.

### 4.2.2 Bioconcentration

Several species of macroalgae and vascular plants have been extensively used for monitoring in many systems as a result of their ability to accumulate metals (Williams 1970; Keeney *et al*. 1976; Harding and Whitton 1981; Whitton 1984).

#### 4.2.2.1 Ecological relevance

Since macrophytes concentrate metals in their tissues, they can, therefore, serve as indicators of environmental metal exposure and contamination (Whitton 1984).

#### 4.2.2.2 Validation

Many studies have investigated the use of macrophytes as indicators of bioaccumulation of metals (Miller *et al*. 1983; Taylor and Crowder 1983a; Campbell *et al*. 1985; Reimer and Duthie 1993). Since the bioconcentration of metals in macrophytes may result from exposure to metals in both water and sediments, making direct relationships between the concentrations measured in the plants and the environment (i.e., water or sediments) can be difficult (e.g., Campbell *et al*. 1985; Mortimer 1985).

A standard method of using macroalgal populations as a routine monitoring tool has not yet been developed, and further research in this area is required. Experiments with several species of macroalgae have shown interspecies differences in their ability to assimilate metals, and the assimilative capacities for different metals within a species can vary substantially (Whitton 1984; Crowder *et al*. 1989).

#### 4.2.2.3 Site specificity

Species composition at any given site will depend on physical and biological factors, and bioaccumulation will depend on the species of macrophyte and the type of metal encountered (e.g., Hutchinson *et al*. 1975). Hence, the effectiveness of using natural macrophytes as a monitoring tool will be site-dependent.

#### 4.2.2.4 Applicability

Because of their life history, aquatic plants are useful for providing an integrated picture of pollution, especially in systems where discharges are intermittent or variable and, as a result, where levels of contaminants in the surrounding waters are also variable (Say *et al*. 1981). A different approach to using metal bioconcentration in macrophytes involves analyzing metal concentrations in macrophytes in conjunction with metals in sediments in order to map bioavailable amounts of metals in lakes and shallow coastal areas (Greger and Kautsky 1993).

#### 4.2.2.5 Practical limitations

The use of a certain species of macroalga may be restricted by the seasonality of its growth, with material suitable for harvesting being present for only half the year in temperate regions. The sensitivity of certain species to metals make them unsuitable as monitors for metal assimilation (due to their absence in metal-rich waters); however, such species can be used as indicators of exposure by their presence or absence in receiving waters. In the latter situation, knowledge of species expected to grow in these waters (as determined from reference-area populations or pre-impact data) and their expected sensitivity to a given metal of concern is required. The fact that macroalgae are capable of adapting to the presence of metals may impose certain limitations to the study. Ideally, a monitoring program involving attached plants will include both macroalgae and periphyton. This flexibility will usually ensure that one or more suitable species is available during most of the year in rivers and in shallower parts of lakes (Whitton 1984).

The choice of one or a set of indicator species of metal exposure must be based on a set of criteria: representative of the study area; widely distributed; and, easy to identify and collect. Because the bioaccumulation of metals in macrophytes is species specific, may be site specific, and is dependant on the concentration of the bioavailable fraction in both the sediments and water column, the choice of the indicator must be based on the type of metal contaminant involved and the known sensitivity of the macrophyte assemblage.

#### 4.2.2.6 Interpretability

Rooted plants reflect the properties of a combination of the ambient water and sediments. As sediment conditions may reflect both historical effluent quality and current effluent characteristics, interpretation of the data may be confounded, especially if the effects of current effluent quality are being monitored. On the other hand, these data would provide relevant information as to the current status of the receiving environment.

Differences in allocation of specific metals between the different organs of macrophytes (i.e. roots, shoots, stems) have been observed. Variations in concentration factors of different metals between roots and shoots (Miller *et al*. 1983) and root and rhizome tissue (Reimer and Duthie 1993) were observed within the same species. Possible explanations for this variability include: the existence of distinct microhabitats within a sampling site; the collection of specimens of differing age; and the sampling of plants at different stages in their growth cycle (St-Cyr and Campbell 1994). Consequently, inconsistent sample collection techniques and preparation may drastically alter the true effects.

Correlations between metal concentrations in plant tissues and sediments are difficult to interpret. In fact, a lack of any relationship between these two variables is common (Campbell *et al*. 1985). A possible explanation for this lack of relationship is that most of this work examined the total metal concentrations in sediments rather that the bioavailable portion of metals in the sediment. More recent investigations have found that total metal concentrations in sediment overestimate the actual bioavailable metal concentrations (Cambell and Lewis 1988; Adams *et al*. 1992). Hence, elevated total metal levels in sediments do not necessarily correspond to higher plant tissue concentrations. The importance of examining the bioavailable portion of the total metals in sediments is now being recognized; however, determining this is not so straightforward, and the extraction procedures and equations used to estimate the bioavailablity of the metals are still being researched. Nonetheless, bioconcentration values can still be useful to demonstrate and monitor the extent of (bioavailable) metal pollution in aquatic ecosystems.

### 4.2.3 Biochemical indicators

Biochemical responses of plant cells can yield information on the effects of metals on aquatic organisms and give an indication of potential stress associated with metal exposure. Detection and measurement of phytochelatin production and different enzyme activities could be used to detect stress before any major impact at the ecosystem level is manifested (NRCC 1985).

Phytochelatins are metal-binding proteins thought to be found in most plants. They are believed to parallel the function of the metallothioneins found in animals, since they both complex trace metals in the cell cytosol. They do differ, however, in both structure and biosynthesis (Couillard 1997). Phytochelatin production has mostly been studied in terrestrial plants and further studies are still needed to confirm their presence in many types of aquatic plants (Rauser 1990). The use of these proteins as biomarkers of metal stress is limited by a lack of information on the extent of their presence in macrophyte species and the costs involved with the collection and laboratory analyses required to determine their production.

Another biochemical response to metal pollution that has been found in higher plants is the induction of the peroxidase enzyme. Van Assche and Clijsters (1990b) developed a biological test system that examined changes in isoperoxidase activity to evaluate the toxicity of metal-contaminated soil, and Byl *et al*. (1994) investigated the use of peroxidase activity in higher plants as a sublethal toxicity test. As with phytochelatins, further research is required before this potentially useful biochemical technique could be used in a monitoring program.

## 4.3 PHYTOPLANKTON

Planktonic microalgae (or phytoplankton) have long been shown in laboratory experiments to be sensitive to various levels of metals, though the development of tolerances to metals has also been observed for many species (Whitton 1984). Very few cases in the literature report on the effects of metal pollution on indigenous aquatic organisms, and, of those that do, the effects on microbiota are usually overlooked in lieu of other components of the aquatic biota, principally fish. There are a few field studies that have considered the effects of metal contamination on phytoplankton, and these form the basis of our evaluation of employing phytoplankton as a monitoring tool for the mining industry. There are several key features of phytoplankton that could make them beneficial as sentinel organisms (key role in trophic chain, high metal concentration factor, abundant and widespread) which are considered in our evaluation.

### 4.3.1 Species composition

The structure of the phytoplankton community (i.e., its species composition) can be altered as a result of environmental stressors that affect certain, more sensitive species. The assessment of alterations in the species composition of natural phytoplankton communities has been widely used to assess the impacts of metal pollution and has been applied to long-term monitoring programs at mine sites (Golder 1996).

#### 4.3.1.1 Ecological Relevance

According to Schindler (1987), the existing approaches for monitoring stressed aquatic ecosystems are inadequate, and he suggests that such a measure as species change in phytoplankton communities may serve as an early indicator of stress and would be suitable as a biological monitoring tool. In fact, this approach has been utilized successfully to examine the effects of metal contamination at several sites (Yan 1979; Austin and Munteanu 1984; Oliveira 1985). Monitoring programs that have examined the structure of phytoplankton communities have also been able to follow the recovery of the receiving aquatic environment (Bonacina *et al*. 1988; Havas *et al*. 1995), as well as the subsequent impacts from other sources (Deniseger *et al*. 1990).

#### 4.3.1.2 Validation

Long-term *in situ* experiments have clearly demonstrated the effects of metal enrichment on the composition of natural phytoplankton communities (Gächter and Máreš 1979; De Noyelle *et al*. 1980). The results of these experiments concur with observations made in field studies.

#### 4.3.1.3 Site specificity

It is usually very difficult to transfer species-specific effects from one water body to another. Though many studies have shown similar effects of metal (and related acid) pollution on phytoplankton communities, the actual species affected are typically site-dependent and will be influenced by a suite of local limnological factors. Therefore, evaluating the use of phytoplankton communities for a monitoring program should be made on a site by site basis. Multivariate statistical techniques can be applied to determine the local influential limnological factors (see below); however, either a spatial or temporal reference data set is required in order to make suitable comparisons with the natural, unaffected system and to properly interpret the data in a local context.

#### 4.3.1.4 Practical limitations

There is a limited resource of experienced microalgal taxonomists available to identify and count the algae in phytoplankton samples. As a result, turn around time for the results and the considerable expense for taxonomic work must be considered.

#### 4.3.1.5 Interpretability

Results from the investigations of the receiving environments of several mine sites suggested that gradients in phytoplankton species (and class) composition were detectable across several bodies of water (Austin and Munteanu 1984; Nicholls *et al*. 1992). Such examples illustrate the potential use of phytoplankton to not only monitor the presence of impacts but also the spatial extent and intensity of impacts.

Though diversity indices, such as the Shannon-Weiner index, and indices of evenness have been used in a few studies where phytoplankton communities have been investigated, the variability in their ability to demonstrate community impacts makes these technique less useful. In some cases, they were capable of representing changes (typically reduction of taxa) to the structure of phytoplankton communities (Thomas and Seibert 1977; Austin and Munteanu 1984), whereas in others, the proliferation of resistant taxa resulted in little or no effect on these indices (Gächter and Máreš 1979; Oliveira 1985), or the level of metal contamination was too low to cause impacts (Findlay *et al*. 1996). A simple quantitative and qualitative examination of species richness will likely provide the most relevant information in biomonitoring programs.

Multivariate statistical techniques (e.g., canonical correspondence analysis) have been used to infer the influence of various limnological variables (including metal concentrations) on community composition (Dixit *et al*. 1989, 1991; Nicholls *et al*. 1992). Such analyses have proven useful to illustrate the effects of acidity, rather than metal concentration, on phytoplankton communities inhabiting metal-contaminated environments. Data from a large number of lakes, however, are required for this statistical technique to be valid.

*Indicator species*

Several commonly found species (or genera) of algae are known to be metal tolerant or metal sensitive (as described in the foregoing studies and in chapter 2 and listed in de Filippis and Pallaghy (1994)). As such, any program examining community structure should also consider the presence/absence of potentially useful indicator taxa. We remind the reader, however, that the presence or absence of such taxa can only be properly interpreted when compared with a reference community. This is especially important considering the ability of known sensitive species to develop tolerance to metals.

Because of the ability of algae to develop tolerance to metals, the interpretation of community data from longer-term monitoring programs needs to be examined carefully. Several examples of such programs have illustrated the presence of species in metal-contaminated waters that are typical of unpolluted waters (Moore *et al*. 1979), and one study even observed the reappearance of intolerant species, which had been previously eliminated, when metal levels were still well above background levels (Deniseger *et al*. 1990).

Another factor that may confound the interpretability of community data in metal-contaminated sites is the effect of low pH on certain species. Metal contamination at most sites is usually associated with elevated acidity, and some studies have in fact found that acid-tolerant rather than metal-tolerant species dominate the affected community (Yan 1979; Nicholls *et al*. 1992).

### 4.3.2 Density and biomass

The density (or standing crop) and biomass (usually measured as Chlorophyll *a*) of phytoplankton communities are typically assessed in any study in which primary producers are considered, and they have been monitored in long-term environmental monitoring programs at mine sites (Golder 1996).

#### 4.3.2.1 Ecological relevance

The dynamics of the response of biomass to metal enrichment makes this variable difficult to interpret in terms of its ecological importance. As often found, phytoplankton biomass will be depressed during the initial exposure period; however, shifts in the species composition of the community towards more tolerant species, as well as decreased grazing by zooplankton, can counteract the initial trend of a lowering of phytoplankton biomass (Thomas and Seibert 1977; Gächter and Máreš 1979; Austin and Munteanu 1984). On a long-term basis, biomass does not appear to be a useful measure of the effects of metal contamination on the primary producers of lake ecosystems.

#### 4.3.2.2 Validation

Experimentation supports field observations of effects of metal contamination on phytoplankton communities: short-term experiments have shown that metal enrichment will reduce the standing crop and biomass of phytoplankton (Côté 1983; Takamura *et al*. 1989; de Filippis and Pallaghy 1994; Havens 1994), whereas on a longer-term basis, densities and biomass are generally similar to those of reference conditions (Thomas and Seibert 1977; Gächter and Máreš 1979).

#### 4.3.2.3 Applicability

The measurement of density and biomass is easily performed and can provide some insight into ecological effects of mine-related effluent; however, the adaptability (or apparent recovery) of these variables following the onset of contaminant exposure suggests that other (preferably, community-level) variables should be measured concurrently. In other words, a qualitative descriptor of density and biomass is also desirable.

#### 4.3.2.4 Interpretability

Biomass and density have been shown to be effective in monitoring the effects of severe metal pollution in lakes (Moore *et al*. 1979; Oliveira 1985; Cabeçadas and Brogueira 1987; Havas *et al*. 1995); however, biomass and density are two variables that can be difficult to interpret when monitoring moderate to low levels of toxicant pollution and, in the absence of community composition information, can actually lead to erroneous conclusions. Yan (1979), for example, demonstrated that phytoplankton biomass in metal polluted lakes will remain similar to that of reference areas. His work also serves as a reminder of the substantial variability of biomass that is experienced by phytoplankton communities over very short periods, as well as over seasonal and yearly time lines. Zooplankton populations can be substantially reduced due to exposure to metals, and, as a result of decreased grazing pressure, phytoplankton density and biomass can actually increase (Roch *et al*. 1985). Moreover, there does not appear to be any relationship between levels of contamination and biomass (e.g., Golder 1996), even in adjacent and similar bodies of water (Moore *et al*. 1979).

### 4.3.3 Bioaccumulation (Metal composition)

The capacity of algae to assimilate metals and produce internal concentrations far greater than those in their surrounding water has been demonstrated in a large number of studies (See chapter 2). Measuring the metal concentrations within algae may provide valuable information about contamination levels in their surrounding water (Whitton 1984).

#### 4.3.3.1 Ecological Relevance

The ability of plants to bioconcentrate metals not only increases the sensitivity of metal detection in the ecosystem, but may also indicate the fraction of the metal in the environment likely to affect the biota. Direct chemical analysis of water will not provide this information. It is worth noting, however, that biomagnification, in the classical sense of the term and as found for organic pesticides and chlorinated hydrocarbons, does not appear to occur with metals (Gächter and Geiger 1979; Förstner and Wittmann 1981; Prahalad and Seenayya 1989; Sanders *et al*. 1989). Since the evidence indicates that metals do not biomagnify through the food chain, but, rather, that they tend to bioconcentrate at a higher rate in algae, these organisms may prove to be the most useful indicators of both bioavailable metals and maximum bioconcentrations likely to be encountered in the aquatic environment.

#### 4.3.3.2 Validation

Metal assimilation by algae has been extensively studied under laboratory and *in situ* experimental conditions; however, many of these studies have found that the relationship between algal metal content and external concentrations is not linear (Cain *et* *al*. 1980; de Filippis and Pallaghy 1994). Though the ability of algae to assimilate metals has been shown many times over, it can be difficult to relate metal concentrations in the cells to those in the environment.

#### 4.3.3.3 Applicability

Algae, as well as other aquatic plants, are useful for providing an integrated picture of pollution, especially in systems where discharges are intermittent or variable, thus resulting in variable levels of contaminants in the surrounding waters (Say *et al*. 1981).

#### 4.3.3.4 Interpretability

Studies using whole phytoplankton communities to measure bioconcentration must consider the community structure. As the assimilative capacity of algae appears to be species-specific, the dominant species of a community will likely influence the bioconcentration values of a phytoplankton community (Denny and Welch 1979), thereby making spatial and temporal comparisons difficult to interpret.

### 4.3.4 Physiological indicators

Metals have been documented to inhibit the growth and photosynthesis of algae (e.g., Gächter and Máreš 1979; Côté 1983). As a result, such physiological indicators as photosynthesis (as determined by $^{14}C$ productivity measurements) have been used as a tool to monitor the long-term effects of mine discharges to aquatic ecosystems (Golder 1996).

#### 4.3.4.1 Ecological Relevance

Results of primary production experiments appear to reflect the physiological status of stressed phytoplankton communities that have been exposed to mine-related contaminants. Moreover, photosynthesis appears to be a more sensitive measure of the effects of metal contamination than biomass in natural populations of phytoplankton (Gächter and Máreš 1979; Côté 1983).

4.3.4.2 Validation

The approach of utilizing primary production rates as a tool to assess the physiological status of phytoplankton communities has been successfully employed in several studies (McKnight 1981; Coté 1983; Cabeçadas and Brogueira 1987). Long-term *in situ* experiments have similarly demonstrated the adverse effects of metal enrichment on the photosynthetic activity of natural phytoplankton communities (Gächter and Máreš 1979; Takamura *et al*. 1989), and short-term experiments have also shown that metal enrichment will reduce the production of phytoplankton (Côté 1983; Takamura *et al*. 1989; de Filippis and Pallaghy 1994; Havens 1994).

4.3.4.3 Practical limitations

Though production may be a more sensitive end-point of exposure to metals than growth, the logistical requirements of using radio-tracers and conducting incubations *in situ* or in a flow-through system must be taken into account for its potential use as a monitoring tool. The expense of employing primary productivity techniques could be prohibitive in low-budget projects; however, production could be measured with the oxygen method, which does not require the use of radio-tracers.

4.3.4.4 Interpretability

The limitations of using density and biomass measurements also apply to the use of primary production measurements in monitoring programs. Community composition (see below), seasonal variability of primary production and grazing pressure will affect the measured rate of primary production of phytoplankton communities.

Primary production studies must consider the photosynthetic capacity of the planktonic communities. Though community-level productivity may be depressed in exposed communities, the photosynthetic capacity (production normalized to chlorophyll *a*) of a community may in fact remain unchanged (Cabeçadas and Brogueira 1987), thus reflecting the shift in species composition toward more resistant species often found in stressed communities. In this particular case, biomass (as measured by chlorophyll *a*) was a more sensitive indicator. Hence, primary productivity reflects the physiological status of the community as a whole, but it does not necessarily represent the physiological status of the organisms making up that community. The issue of adaptability must, therefore, also be considered in interpreting monitoring programs using physiological indicators as monitoring tools. Most programs do not examine the data to this level, and, as a result, photosynthetic rates may not reveal any significant patterns (Golder 1996).

### 4.3.5 Biochemical indicators

The photosynthetic pigments in phytoplankton communities have been shown to undergo various changes as a result of exposure to contaminants (Rai *et al*. 1981; Singh and Rai 1991; de Filippis and Pallaghy 1994). Further research is required, however, to understand the physiological implications of these alterations and the resulting influence on community structure before these analyses can be utilized as monitoring tools.

Phytochelatins have been shown to be the major intracellular metal binding peptides of plants, algae and some fungi (Rauser 1990). Many metals have been shown to induce the production of phytochelatins, which function analogously to the metallothioneins observed in fish and other animals exposed to metals (Grill *et al*. 1987). The production of this peptide in plants is believed to be part of a general metal detoxification system, although certain metals (especially Cd) have been found to be far better inducers of phytochelatin synthase activity than others (e.g., Zn, Co and Ni) (Ahner and Morel 1995).

Alkaline phosphatase is an enzyme that catalyses the hydrolysis of dissolved organic phosphate in phytoplankton. Its activity was found to be inhibited by copper, and, as a result, has been suggested as a potential biomarker of metal toxicity (Rueter 1983). Though the measurement of alkaline phosphatase activity is relatively simple and the ecological relevance of its inhibition potentially important, considerably more study is required to consider it for use as a monitoring tool.

#### 4.3.5.1 Ecological relevance

Alteration of photosynthetic pigments and the induction of phytochelatin are both promising techniques that could be used as early warning systems of metal contamination. Phytochelatin induction is especially promising as an early indicator of metal exposure since production of this peptide is often induced at metal concentrations that do not affect growth. The ecological relevance (e.g., relationship between community health and phytochelatin induction) of increased phytochelatin production has yet to be investigated.

#### 4.3.5.2 Validation

At present, the number of studies that have examined changes in pigment composition due to metal contamination is insufficient to recommend such an approach to monitoring programs.

Besides one known study that examined phytochelatin induction in natural marine phytoplankton communities (Ahner *et al*. 1994), phytochelatin studies with algae have only considered cultures of marine species; thus, further studies are required before this potentially useful technique is employed in biomonitoring programs.

#### 4.3.5.3 Applicability

Phytochelatins, in particular, could serve as useful indicators of exposure to metals.

#### 4.3.5.4 Practical limitations

The procedure for analyzing photosynthetic pigments is well established, is straightforward and allows for the simultaneous analysis of many samples.

In contrast, the preparation required for the analysis of phytochelatin production is very involved and sensitive and would require a laboratory specially equipped (and trained) for such analyses. The recent development of this approach suggests that only a few laboratories conduct such analyses.

#### 4.3.5.5 Commercial availability

Laboratories specially equipped to perform assays for phytochelatin synthase activity would have to be found, and the cost of such assays would, at present, likely be prohibitive for a multi-sample monitoring program.

### 4.3.6 Size spectrum analysis

The approach of enumerating various phytoplankton size classes and estimating their biomass is based on theoretical considerations of the distribution of body sizes of aquatic organisms (Platt and Denman (1978) as cited in Munawar and Legner (1993)). By using a technique that transforms size data into normalized curves, Sprules and Munawar (1986) were able to reveal perturbations, in the form of increased variability in size groups, to a plankton community in the Great Lakes.

#### 4.3.6.1 Ecological relevance

The relationship between size spectra alteration and ecological importance is in need of further research. This approach, however, can be used as a warning system of stress to phytoplankton communities and, potentially, to the aquatic ecosystem.

#### 4.3.6.2 Validation

Very few laboratory and microcosm experiments have analyzed the effects of metals and other contaminants on the size spectrum of algae thus far, though a general pattern of decreased cell size in exposed phytoplankters has been observed (Sanders and Cibik 1988; Munawar and Legner 1993).

#### 4.3.6.3 Practical limitations

This approach is lab-intensive and, to be efficient, requires sophisticated computer-based equipment. Less costly equipment could be used; however, the time requirements for measurements could be prohibitive, especially in a monitoring application, where multiple samples would be required (see below). This method is in the developmental stage and is being applied for some programs (Munawar and Legner 1993).

#### 4.3.6.4 Interpretability

The level of metal exposure in natural environments required to trigger a size distribution response is not known, making any interpretation of metal effects difficult. Other stressors could also lead to detectable effects on size distribution. As with all other methods, multiple sampling periods are required due to the seasonal variability of community composition, and additional data from reference (spatial and temporal) samples would be required for comparative purposes.

### 4.3.7 Tolerance

A recent study has successfully used the tolerance of phytoplankton communities to test the exposure of these communities to arsenate and copper contamination, which originated from a smelter (Wängberg 1995). The system used in this approach was introduced by Blanck (1985), who examined the tolerance of periphyton communities as indicators of exposure to arsenate (Blanck and Wängberg 1988b). This technique as a tool for phytoplankton is at its infancy and certainly requires, and merits, further examination. Further details of this approach as a potential monitoring method for metal contamination are presented in the Periphyton Section of this report.

### 4.3.8 Morphological indicators

As described in chapter 2, deformation of diatom frustules has been observed in algae exposed to elevated levels of metals. This effect has been observed in both natural and laboratory settings, and microscopic observations are easily made.

#### 4.3.8.1 Ecological relevance

The ecological relevance of frustule deformities or any other, associated adverse effects are not known. Such morphological abnormalities could be used as indicators of exposure to metals; however, other chemical characteristics, which can be associated with metal contaminated waters, have also been found to cause deformations in diatoms (Barber and Carter 1981). Furthermore, there appears to be a natural occurrence of diatom deformities, a phenomenon that requires further documentation. Further investigation into the ubiquitousness of this response to contaminants and other factors is warranted before adopting this technique into monitoring programs.

#### 4.3.8.2 Practical limitations

The equipment and sample processing required are straightforward, though detection of slight deformities requires specialized equipment (e.g., scanning electron microscope).

## 4.4 PERIPHYTON

Periphyton is defined as a complex microcommunity developing on submerged natural and artificial substrates (Weitzel 1979). It occupies an important niche in most freshwater systems as it provides reduced carbon compounds to higher trophic levels. Periphyton can, therefore, influence the rest of the aquatic ecosystem (Whitton 1984). Periphyton can be used as an indicator to detect or forecast impacts at some level of biological organization, from biochemical to ecosystem responses (Hellawell 1986). The microalgal component of periphyton can be used to monitor the bioaccumulation of metals (i.e. as an indicator of metal exposure) or as an indicator of ecological effects. Both of these approaches are discussed in terms of their suitability as monitoring tools to detect potential effects from mining effluents.

### 4.4.1 Structural and functional measurements of periphyton

Biomonitoring studies examining the impacts of metals on periphyton have focused on structural measurements and functional changes at the community level. Structural measurements generally involve counts of organisms comprising a system (providing information on abundance, species richness, community structure), whereas functional measurements study changes in primary productivity, respiration and detritus processing (Clements 1991).

#### 4.4.1.1 Ecological relevance

It can be argued that, in contrast to any other level of organization, examining periphyton at the community level provides the most relevant ecological information about ecosystem impacts. Broad taxonomic groupings or particular sensitive species can be assessed in terms of general composition and abundance; however, it is often difficult to obtain representative and uniform samples because of spatial heterogeneity and difficulty in sampling these organisms (Weitzel *et al*. 1979). A more practical measure involves the study of changes in species richness and diversity, since there are different types of mathematical indices (e.g., community diversity index (Shannon 1948), species evenness index (Pielou 1966) and other biotic indices (Descy 1979)) that may be used to assess the species diversity and richness of a community (Lafont *et al*. 1988). More recently, entire community inference models based on canonical correspondence analysis (Cattaneo *et al*. 1995; Lowe and Pan 1996), a multivariate statistical ordination technique that arranges sites along axes according to species composition, have been used with variable success (Cattaneo *et al*. 1995).

#### 4.4.1.2 Validation

Many studies that have characterized the algal communities of streams have related the abundances of specific taxa to the presence of metals (Clements 1991). Other studies have looked at changes in abundance of different species following exposure to metals (Whitton 1984). Reduced species diversity and richness appear to be the most common effects, with the abundance of species sensitive to metals generally decreasing, being replaced by more tolerant ones (Austin 1983; Austin *et al*. 1985; Austin and Deniseger 1985; Deniseger *et al*. 1986). Diatoms are a sensitive taxa in periphyton since some species will tend to decrease in abundance under stress. Filamentous green or blue-green algae are frequently observed in polluted sites and are generally more tolerant to metal-induced stresses. (Patrick 1978; Weber and McFarland 1981; Weitzel and Bates 1981).

Biomonitoring studies investigating the impacts of metals on periphyton have mostly focused on structural measurements rather than functional changes in these communities (Clements 1991). Crossey and La Point (1988) compared the use of both types of community measurements to observe effects of metals on periphyton. Functional measurements included community respiration, gross primary productivity, chlorophyll *a* content and ash-free dry mass (AFDM), all of which were greater at impacted and recovery sites than at reference stations. In a study designed to evaluate direct toxicity and food-chain effects in aquatic systems, Boston *et al*. (1991) also used functional measurements (such as algal biomass (chlorophyll *a*) and photosynthetic rates) of periphyton communities.

Effects of metals on both structural and functional responses of periphyton have also been observed experimentally (Leland and Carter 1984; Genter *et al*. 1987; Clements 1991). The use of artificial substrates to collect periphyton (Lowe and Pan 1996) is one approach that has been used since it permits the standardization of external factors such as light, current and substratum among sites. The use of artificial streams (i.e., mesocosms) makes it possible to control many of the extraneous variables, such as light, currents, grazers and so on, that can play a role in community changes and hence confound results (Weber and McFarland 1981; Lowe and Pan 1996).

#### 4.4.1.3 Site specificity

The diversity of aquatic ecosystems that support periphyton communities does not allow us to make generalizations as to the influence of metals on periphyton species and community composition. Ranging from lakes to fast-flowing rivers, these aquatic environments will differ in their nutrient loads, light regimes, grazer communities and water quality characteristics, all of which have been shown to affect periphyton communities (Kutka and Richards 1996). Substratum type can influence species composition (Stevenson and Hashim 1989), and water velocity and light levels will influence both colonization rates and assemblage composition (Stevenson 1983). Consequently, a general monitoring program should select indicator species based on site- and metal-type-specific bases.

#### 4.4.1.4 Applicability

Most of the biomonitoring programs that have used periphyton have investigated the impact of point-source contaminants on lotic systems, though periphyton can also be used to monitor long-term changes in aquatic communities, in both lotic and lentic systems, resulting from changes from non-point sources (Lowe and Pan 1996). Periphyton possesses many attributes that make it a suitable indicator of current environmental conditions, because:

1. periphyton is primarily composed of autotrophs; therefore, it occupies an important niche (that of primary producers) in most freshwater ecosystems (Whitton 1984);
2. periphyton is usually species-rich and spatially compact relative to other aquatic groups, and representative samples can be collected from a few square centimeters of substratum (Lowe and Pan 1996); and,

3. organisms within periphyton have relatively high turn-over rates; hence, they are among the first organisms to respond to environmental stress and among the first to recover. As a result, they typically reflect current environmental conditions (Lowe and Pan 1996).

#### 4.4.1.5 Practical limitations

*Functional measurements*

Functional measures have been described as providing an important insight into how community metabolism responds to contaminant inputs and how changes in community structure affect community metabolism (Crossey and La Point 1988); therefore, functional parameters can describe the ecological consequences of ecosystem changes. These measures, however, require laboratory equipment, large sample numbers and considerable sample preparation, all of which could lead to prohibitive costs, especially if the periphyton component of the monitoring program is combined with the more traditional benthic macroinvertebrate and fish surveys.

*Structural Measurements*

Assessments of community structure in monitoring programs have clearly demonstrated that the species composition at sites impacted by metals can be different than that at reference sites (Clements 1991). Although these measures can yield ecologically-relevant information, they are time-consuming and could be limited by taxonomic difficulties. For instance, it is necessary to enumerate a minimum of 50 to 100 cells per sample (Round 1991), though most researchers enumerate from 300 to 500 organisms due to the large number of species. Since a typical diatom community in an unpolluted habitat may consist of hundreds of species occupying a relatively small area, the taxonomic expertise required to identify periphyton to the species level may prohibit the use of these organisms for certain routine biomonitoring programs in streams (Lowe and Pan 1996).

#### 4.4.1.6 Interpretability

*Functional measurements*

Of all the functional measures that have been employed thus far, biomass can be the more complicated to assess. Changes in biomass have also been used as indirect estimates of productivity which is appropriate in situations where biomass loss through grazing is minimal. In systems where eukaryotic filamentous algae dominate, wet weights provide a suitable estimate for algal biomass. But when community composition varies among treatments, dry weight (DW) or ash-free dry weight (AFDW) are better estimators of community biomass. It can also be estimated by chlorophyll *a* concentrations; however, a direct measure (DW, AFDW) of biomass is more reliable (Lowe and Pan 1996).

*Structural measurements*

The sample size, the number of replicates and the statistical analysis used to test for differences between sample sites are important factors that can greatly influence the outcome of an assessment for impacts. Reference sites and impacted and recovery sites should be similar in all respects, except for the presence of the contaminant in question; however, the difficulty in locating comparable sites due to natural changes in structural and functional parameters along stream gradients (such as stream velocity, current, etc.) is a confounding factor that can greatly influence the data (Clements 1991).

A lack of pre-impact information will decrease one’s ability to associate a causal relationship to any impacts observed. This is important because the goal of many monitoring programs is to establish such a link, which can be very difficult if the receiving stream receives discharges from several different sources.

*Artificial substrates*

The principal advantages of using artificial substrates is in the interpretation of the results since these substrates can be manipulated to examine very precise locations with regards to a discharge, thereby reducing potential confounding factors. Other influential factors are also best controlled with artificial substrates: the homogeneity of the material eliminates natural substratum influences, and the replication possibilities can help reduce the high variability typically found among natural samples (Weitzel *et al*. 1979).

### 4.4.2 Bioaccumulation

Concentrations of metals in periphyton are sometimes used as indicators of the exposure of organisms to metals in streams, although, as described above, the more common use of periphyton as an indicator consists of measures of species diversity and richness. Organisms sampled directly from aquatic systems, from artificial substrates or from experimental streams have been used to assess the assimilation of metals by periphyton communities.

#### 4.4.2.1 Ecological relevance

Though several studies have found a relationship between metal concentrations in water and plants (Kelly 1988), levels in periphyton may be several orders of magnitude greater than concentrations in surrounding water due to the ability of aquatic plants and the algal component of periphyton to bioconcentrate metals. This can have important consequences to the entire ecosystem because, in their role in energy transfer in aquatic systems, periphyton represent an important link in the transfer of metals to higher trophic levels (Newman and McIntosh 1989).

The use of periphyton as an indicator of metals requires knowledge of the relationship between concentrations in the organisms and in the surrounding water, in addition to the environmental factors that may influence this relationship. As a result, this approach can be more complicated than determining basic species diversity; however, when the concentration of metals in the surrounding water approximates detection limits, it may be more practical to analyze concentrations in periphyton (Clements 1991).

#### 4.4.2.2 Validation

Several researchers have suggested that periphyton be used for the biomonitoring of metals (Ramelow *et al.* 1987; Newman and McIntosh 1989). Although it is generally assumed that the intracellular concentrations of metals in algae and periphyton result from biological uptake, results of bioaccumulation studies with these organisms should be interpreted cautiously since both biotic and abiotic components of the periphyton community (referred to as aufwuchs) can concentrate metals (Newman and McIntosh 1989). Furthermore, metals associated with the abiotic portion of periphyton may not be available for transfer to higher trophic levels (Clements 1991). Because of the underlying problems associated with the use of periphyton as an indicator of metal exposure and concentrations in the aquatic system, most studies have concentrated on measures of species diversity and richness.

#### 4.4.2.3 Interpretability

The relationship between burdens (and, hence, implied toxicity) and ambient concentration of metals has been found to be metal specific. Several variables influence the toxicity (and bioavailability) of metals to aquatic biota, including speciation, type and concentration of available ligands and type and concentration of dissolved cations ($Ca^{2+}$, $Mg^{2+}$, $H^{+}$) that compete with metals for binding sites on organism surfaces (Brezonik *et al.* 1991). Work undertaken by Brezonik *et al.* has shown that increased acidity may even reduce the burden of certain metals. As a result, interpreting the link between metal burdens in periphyton and exposure is very difficult.

### 4.4.3 Laboratory ecotoxicological test system

This periphyton test system was originally designed to measure short-term effects of exposure to arsenate on photosynthesis (Blanck 1985). A more recent study using this system tested whether inhibition of periphyton photosynthesis in short-term experiments was relevant for a prediction of longer-term effects on species composition and net community production (Blanck and Wängberg 1988b). In this study, periphyton communities were sampled by submerging artificial substrates in the natural waters of a fjord and in a flow-through indoor aquaria system, where specific contaminant concentrations are manipulated. Water for the aquaria was obtained from this same fjord. Photosynthesis experiments and the determination of biomass and species composition were undertaken on periphyton samples collected from both the fjord and the aquaria.

#### 4.4.3.1 Ecological relevance

Though most short-term test systems are incapable of generating ecologically relevant information, the ecotoxicological test system presented by Blanck and Wängberg

(1988b) works under the premise that effects on metabolism are indicative of changes in structure and production and, hence, lead to long-term effects in the community. Though this has been demonstrated in the laboratory (Blanck and Wängberg 1988b), it still remains to be shown whether short-term metabolic effects in the laboratory can predict long-term community-level effects in natural ecosystems. Ecotoxicological test systems can be used, however, as an early warning indicator of metal-specific effects on periphyton communities.

#### 4.4.3.2 Validation

Blanck and Wängberg (1988b) demonstrated that short-term effects of arsenate on photosynthesis in periphyton grown in the laboratory can predict those in periphyton cultured in natural ecosystems. They also demonstrated that short-term photosynthetic responses to arsenate could predict long-term biochemical, physiological and community-level changes in the laboratory-grown periphyton; however, the predictive value of this periphyton test requires further validation by experimenting with different chemicals.

#### 4.4.3.3 Site specificity

Though most laboratory-based tests do not represent the local conditions of the area of concern, the laboratory ecotoxicological test system does use the periphyton community from the actual receiving environment.

#### 4.4.3.4 Applicability

The ecotoxicological test system could be used to identify potential impacts of certain metals to a given ecosystem; however, it is not suited for long-term, post-impact monitoring programs. This system could also be used to monitor seasonal and geographical variations in the periphyton community’s sensitivity to certain compounds.

#### 4.4.3.5 Practical limitations

As the flow-through aquaria require ambient (or receiving environment) water, such a testing technique would require considerable expense and effort. A minimum of 3 weeks would be required for the artificial substrates in this system to be adequately colonized.

#### 4.4.3.6 Interpretability

Since periphyton communities are established on artificial substrata in a short (e.g., 3 weeks) period of time, this approach is likely to select mainly for opportunistic species. This was demonstrated in Blanck and Wängberg's (1988b) study by the substantially different communities that colonized the *in situ* versus the laboratory substrata. Colonization time and selectivity are two factors that could influence the sensitivity of periphyton to certain chemicals and, hence, confound the interpretation of the ecotoxicity of xenobiotics in this system.

### 4.4.4 Pollution-induced tolerance

Pollution-induced tolerance may be used to assess the impact of pollutants in aquatic environments and to identify the chemical(s) causing the impact. This premise was proposed by Luoma (1977), who suggested that the development of resistance to a trace contaminant by a benthic algal population was direct evidence of a selective pressure response exerted by the contaminant and that resistance to a specific chemical implies that this chemical is the cause of biological impact. This concept can also been applied at the community level of biological organization, where observed community tolerance can be used as direct evidence that a community has been affected and restructured by a toxicant (Harrison *et al*. 1977; Thomas and Seibert 1977; Blanck and Wängberg 1988a).

#### 4.4.4.1 Ecological relevance

Though tolerance may be a useful indicator, it is not very informative if not accompanied by assessments of other responses in individuals, population or communities. Assessing the tolerance of biota at lower levels of organization requires the assumption that other community members are likely to have been affected as well. Assessing tolerance at the community level avoids this assumption and makes use of the interspecific variation in sensitivity rather than just the variation within a species. This is the principal advantage of utilizing such an approach with periphyton.

#### 4.4.4.2 Validation

The concept of induced tolerance as an impact assessment tool requires further verification. The concept that tolerance to contaminants is induceable in algae appears to be clear (Stokes *et al*. 1973; Jensen *et al*. 1974; Harding and Whitton 1976; Stockner and Antia 1976; Gavis et al. 1981; Foster 1982; Cosper *et al*. 1984; Klerks and Weis 1987). Tolerance to metals at the community level in algae has also been demonstrated (Harrison *et al*. 1977; Thomas and Seibert 1977; Blanck and Wängberg 1988a). Induced tolerance at the community level appears to be the most relevant approach in using tolerances as a monitoring tool; however, further work is required to confirm the utility of this method in various types of ecosystems and under the influence of different xenobiotics.

#### 4.4.4.3 Applicability

Induced tolerance in periphyton can be used as a useful ecotoxicological tool and help predict the effects of specific toxicants on natural periphyton communities. This method could be especially useful at determining the chemical responsible for causing an observed change in the periphyton community.

#### 4.4.4.4 Practical limitations

This approach requires a laboratory and access to receiving environment (control) water. A battery of short-term metabolic tests would be required to measure the community tolerance, and a given amount of time would be required for the periphyton communities to develop.

#### 4.4.4.5 Interpretability

The limited number of experiments conducted thus far on tolerance induction on periphyton communities demonstrate that sensitive species were replaced primarily by community members with the highest tolerances (Blanck and Wängberg 1988a). This is beneficial to the detection of tolerance since the most sensitive components of the community have been replaced with the most tolerant ones; in essence, the community dynamics appear to amplify the response to a toxicant.

## 4.5 CONCLUSIONS

Adverse effects as a result of exposure to moderate to high levels of metals can be demonstrated at many levels of biological organization in aquatic plant (macrophyte, phytoplankton and periphyton) assemblages. The task of conclusively proving that observed responses at higher levels of organization (e.g., community) are attributable solely to metal exposure has been shown to be most challenging. Conversely, a response to metals at a lower level of organization (e.g., sub-cellular) does not imply a response at a higher level. Because compensation for metal effects occurs at each level of organization, impairment at a lower level of organization may elicit only compensation at the next level (Luoma and Carter 1991).

Biochemical responses to metals have been intensively studied; however, the ecological relevance of these responses (e.g., bioaccumulation) are rarely considered. Physiological responses in plants (e.g., photosynthesis) have received less attention and are usually influenced by a suite of other factors, and, even at this lower level of organization, differentiating the influence of metals can be difficult. The tolerance of certain populations of plant assemblages have been investigated; however, long-term tolerance to metals may confound any population-specific response previously shown. The large variability typically found in measures of community responses makes conclusions about impacts to metal exposure difficult to ascertain. Moreover, structural changes at the community level may develop too slowly for short-term impacts and warning signals to be manifested.

Ideally, long-term monitoring programs, including pre- and post-impact periods, would be implemented, and such programs would examine several levels of organization concurrently. Of course, such an ideal situation is rarely practical or affordable. In lieu thereof, each receiving environment must be thoroughly examined prior to the implementation of any monitoring program, and only once this baseline information has been digested should the decision as to the monitoring endpoints be made. Various criteria should be considered during this decision making, including:

- natural variability of the target assemblage;
- presence of other contaminants and nutrients in the discharge monitored;
- response to exposure to any such chemicals and/or metals by lower levels of biological organization;
- availability of suitable reference habitat and biota; and,
- influence of any other potential biotic (e.g., species interactions, nutritional status, growth-limiting factors) and abiotic (e.g., temperature, salinity, oxygen, other physical and chemical characteristics) confounding factors.

A summary of characteristics of the aquatic plants herein considered as tools for biomonitoring the effects of mine-related effluents is presented in Table 4.1. These characteristics should be considered during the design phase of any monitoring program that may use aquatic plants.

The quality and, hence, interpretability of any monitoring program will only be as good as the design and execution of a given program. Our experience with assessing long-term monitoring programs in general has revealed that the major limiting factors are inconsistent data collection methods and techniques, inconsistent sampling periods and inconsistent sampling locations, and this was also found to be the case with a long-term monitoring program for aquatic plants at a mine site (Golder 1996). These inconsistencies, coupled with the large natural variability that is typically found with most plant-related variables, limited the amount of useful information from an otherwise extensive and comprehensive data set.

**TABLE 4.1**

**COMPARISON OF AQUATIC PLANTS WITH POTENTIAL FOR USE AS SENTINELS IN BIOMONITORING PROGRAMS FOR THE MINING INDUSTRY [a]**

| INDICATOR | USE | ADVANTAGES | DISADVANTAGES |
|---|---|---|---|
| Phytoplankton; species diversity / richness | Measures of stress at the organism, population and community level | •key role in trophic chains<br>•abundant and widespread<br>•early indicator of ecosystem stress<br>•several species known to be sensitive to metal contamination | •drift (i.e., not sessile)<br>•seasonal growth variability<br>•high sensitivity to various toxicants and local limnological factors<br>•limited availability of taxonomic expertise<br>•proliferation of resistant taxa and development of resistance will mask impact |
| Phytoplankton; bioaccumulation of metals | Measures of metal concentrations | •key role in trophic chains<br>•abundant and widespread<br>•high bioconcentration factor<br>•increases sensitivity of metal detection in the ecosystem<br>•provides integrated picture of pollution | •drift (i.e., not sessile)<br>•changes in community structure affect bioconcentration values<br>•as a result of above, spatial and temporal comparisons are difficult<br>•expensive laboratory analyses |
| Periphyton; species diversity / richness | Measures of stress at the organism, population and community level | •indication of ecological effects<br>•laboratory time required is short<br>•responds quickly to environmental disturbances | •limited availability of taxonomic expertise<br>•susceptible to confounding effects from natural sources |
| | | | |

| INDICATOR | USE | ADVANTAGES | DISADVANTAGES |
|---|---|---|---|
| Periphyton; bioaccumulation of metals | Measures of metal concentrations | •indicator of metal exposure of other organisms<br>•extrapolate to levels in $H_20$ at low chronic levels | •no direct relationship, i.e., bioaccumulate at higher rates than environmental levels<br>•expensive laboratory analyses |

**TABLE 4.1**

**CONTINUED**

| INDICATOR | USE | ADVANTAGES | DISADVANTAGES |
|---|---|---|---|
| Macrophytes; species diversity / richness | Measures of stress at the organism, population and community level | •sessile organisms (representative of surrounding environment)<br>•easy to identify and quantify and does not require extensive expertise<br>•important link to higher trophic levels<br>•longer-term indicator (e.g., seasonal) than phytoplankton (e.g., weekly) | •results may be affected by confounding, natural factors (e.g., light, water flow, etc.)<br>•represents sediment and water conditions - undesirable as indicator of water quality only |
| Macrophytes; bioaccumulation of metals | Measures of metal concentrations in different organs (exposure) | •sessile organisms (representative of surrounding metal levels)<br>•important link to higher trophic levels<br>•longer-term indicators (e.g., seasonal) than phytoplankton (e.g., weekly)<br>•integrated response: over time and sediment and water | •bioaccumulate at higher rates than environmental levels<br>•different organs accumulate metals at different concentrations<br>•concentration of metals is species specific and metal specific<br>•expensive laboratory analyses<br>•represents sediment and water conditions - undesirable as indicator of water quality only |
| Macrophytes and phytoplankton; bioassays | E.g., phytochelatin induction and other enzyme induction | •measure of exposure of organisms<br>•cellular effects may be early signal of potential for larger effects | •expensive laboratory analyses<br>•methodologies not well developed |

a - Only the methods that have been used in or that are readily available for biomonitoring programs are listed. Many other techniques are in the developmental or research stages, and they are described in the text.

# RECOMMENDATIONS

## MACROPHYTES

Further investigations of the relationships between the metal content of rooted submerged macrophytes collected over a contamination gradient and the estimated free-metal ion concentrations of the sediment interstitial water need to be conducted. Plants may be collected at the same time as other more routinely sampled benthic organisms used to monitor the environment, such as aquatic insects and mollusks, which also rely on the free-metal ions at the sediment/water interface as a source of bioavailable metals. In this way, the usefulness of using macrophytes as biomonitor organisms could be objectively evaluated and compared with the use of the more «standard» biomonitor organisms.

Measurements of phytochelatins and peroxidase activity in a few key plant species over a wide gradient of contamination due to mine activities are also suggested.

## PHYTOPLANKTON

Phytoplankton could be advantageously integrated in a monitoring program of mine sites. Many species of phytoplankton are widespread, and when calibrations have been established, they could be probably extended to other sites. Phytoplankton are a choice when monitoring lakes or reservoirs, but they are not suitable for streams where phytoplankton are reduced and for large rivers where they can be abundant but are difficult to interpret because of movement of the water masses. Periphyton is generally a better indicator of local conditions, because of its sedentary nature, while phytoplankton may integrate conditions across larger spatial scales. Phytoplankton are also less practical than periphyton for accumulation studies since there is the added step of concentrating the sample, while filamentous algae are macroscopic.

**PERIPHYTON**

Determining what is to be measured in a biomonitoring program will depend on the sensitivity of the biological parameter to chemical changes. Regardless of whether studies are carried out in experimental streams or in the field, on natural or artificial substrates, methodologies to study structure and function of periphytic communities are similar to those used for phytoplankton. Thus, different tools for biomonitoring presented in the chapter on phytoplankton are, in general, applicable to periphyton. The principal difference between using phytoplankton and periphyton rests in the limitations of sampling methods for periphyton. There is not yet a set of standard methods which are accepted by all researchers. Experimental methods are constrained by different factors such as available time, laboratory equipment, as well as funding. Therefore, it is important for assuring valid comparisons among studies that methods be accurately described and justified.

In this context, the use of artificial substrates is justified for a variety of reasons: to reduce the heterogeneity of the naturally occuring substrate, for a standard means of comparison between two habitats or sites; to decrease cost of sampling; to decrease disruption of the habitat; and to decrease time to obtain a quantitative sample, since the surface area of the substrate is known.

Changes in species or their relative abundance and distribution are sensitive indicators of metal contamination. Such structural measures are relatively inexpensive to monitor. These measures do require specialised expertise, but this is relatively available. On the other hand, community function measures require more equipment and necessitate more replicates in order to estimate variability.

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# APPENDIX

COST-EFFECTIVENESS

In the following pages, some of the most promising monitoring methods using macrophytes, phytoplankton and periphyton are recommended by the authors. However, estimating the cost associated with each of these methods is difficult for many reasons.

1) The proposed methods often come from newly developed ideas, from scientific researchers in universities or governmental institutions, and are thus not commercially available on a routine basis in private laboratories. So, only an estimation can sometimes be done, or addresses indicated where the analysis can be done.

2) The cost of a technician is also difficult to estimate. In universities, work is often done by graduate students. Some methods require only general laboratory technicians, while others need more qualified ones, such as technicians or biologists trained to recognized algae species under a microscope or macrophyte species in the field, or able to lead a field survey and sample collection. When the collection of data is finished, the interpretation of the results must be done by someone very familiar with the problem and able to provide proper conclusions.

3) Field work is also difficult to quantify in terms of costs. It must often be determined on a case by case basis, depending on the size of the area to be surveyed, the number of samples to be collected and the difficulty of collecting the samples, etc..

So, only a «gross» estimation of the time/technician required for each technique, necessary training, the instruments needed, etc... has been indicated for each of the monitoring tools proposed.

## DRAFT FORMAT FOR DESCRIBING MONITORING TOOLS

**MACROPHYTES**

METHOD NAME: Species composition of plant communities.

PURPOSE AND DESCRIPTION: When doing a general survey of the environment surrounding mine activities (tailings, smelting), a list of macrophyte presence/absence, a rough count of the species number and the species encountered can be done. The absence of submerged/emergent plants from streams/water bodies which would normally support them, when compared to control areas not affected by mine activities, or the presence at those sites of certain indicator species recognized to be tolerant, can be regarded as an indication of general pollution due to mine activities. Briefly, it consists to compare the higher plant communities associated with water bodies that are known to be of high quality with those of known degraded system. Such a survey over a large contamination gradient can be done fairly rapidly by biologists trained to recognized plant species.

LIMITATIONS: Since comparison with healthy environments is the basis of the method, the ideal reference site must be similar in all respects, except for the presence of metal contamination and of acidity due to mine activities. Besides acidity and metal contamination, other associated effects of mine activities, such as high turbidity and suspended solids, irregular water levels, rough substrates and poor nutrient levels can also have severe consequences on inhabitants of the affected water bodies.

COSTS: - The cost of the field survey will be proportional to the dimensions of the area covered (the affected territory and control sites).

- At least two technicians, of which at least one is a biologist or a technician trained to recognized macrophyte species; the ability to do scuba diving is an asset.

- Boat + truck, etc...

- The compilation of the data, the statistical analyses and their interpretation would be preferably done by the biologist who has done the field survey.

Useful reference: Small *et al*. 1996.

COMMENTS AND RECOMMENDATIONS: Such a procedure could perhaps be applied at mine sites to determine the area affected by mine activities and to follow in time the restoration of the sites.

**MACROPHYTES**

METHOD NAME: Metal accumulation in plant tissues.

PURPOSE: The metal concentrations in macrophyte tissues reflect the bioavailable metal concentrations in the environment. Analysis of plant tissues presents several advantages over analysis of water or sediment, or of animal tissues. Free-metal ion concentrations in the interstitial water at the water/sediment interface must also be determined.

DESCRIPTION: Rooted submerged species take up most of their metals from the sediment interstitial water; uptake by submerged foliage would be expected to become important when metal concentrations in the surrounding water column are high. Relationships must be studied between the free-metal ion concentrations in the interstitial water/dissolved metals in the water column and the metal concentrations in plant tissues.

LIMITATIONS: Plant parts must be adequately washed before analysis for metal determination to remove external contamination. Within-site and seasonal variability must be accounted for. Previously published equations to estimate the free-metal ion concentrations in the interstitial water from partial extraction procedures to partition metals in sediments among various defined forms, must be refined, and include all parameters affecting metal availablity to plants such as pH, sediment organic carbon, dissolved [Ca], etc...

COSTS: - Completion of the field work; the cost will be proportional to the number of lakes/water bodies to be sampled, the number of replicates per site, the number of plant species analyzed... In the field: two or three technicians, of which at least one is an experimented SCUBA diver, and one is a biologist or a technician trained to recognized macrophyte species. Involves collection of sediment cores, water samples and plants.

- In the laboratory: technician trained to do a sequential extraction procedure on the sediment, to determine the free-metal ion concentrations of the interstitial water at the water/sediment interface. Metal concentrations in the leachates and in the water column can be measured by atomic absorption spectrophotometer (AAS), graphite furnace AAS, or an inductively coupled plasma atomic emission spectrophotometer (ICP), depending on the metal analyzed and of the detection limit wanted.

A sequential extraction procedure is routinely performed in the laboratory of INRS-Eau, at the following address: Université du Québec, Institut National de la Recherche Scientifique, INRS-Eau, 2700 rue Einstein, C.P. 7500, Sainte-Foy, Québec, G1V 4C7. Tel: (418) 654-2524. Stéphane Prémont is head of the laboratory. For 9 sediment samples, extracted into 6 fractions, analyzed for Fe, Mn, Cd, Cu, Pb, Zn and Ni using ICP, the total cost is about $3,000.00.

- The sediment organic carbon, the pH and the water content must also be determined.

Useful references: Tessier 1992, Tessier *et al*. 1979, 1993.

- The dissolved Ca concentrations and the pH of the water column are also useful parameters to be measured.

- Plants must be properly cleaned and analyzed for their metal content. As an example, in the laboratory of INRS-Eau, sample preparation (cleaning, grinding and digestion) = $20.00 per sample. An additional step of cleaning can be necessary if root samples are analyzed. Analysis of Fe, Mn and Zn by ICP is $15.00 per sample (for all 3 metals). Analysis of Cd, Cu, Pb and Ni using graphite furnace AAS (because of the small concentrations) is $15.00 per sample, per metal.

Useful reference: St-Cyr and Campbell 1997 - to be submitted to Can. J. Fish. Aquat. Sci.

- Certified reference materials for plant and total metal concentration in the sediments are commercially available.

- Boat, truck, coolers, SCUBA diver materials, laboratory materials (acids, etc...)

- Data must be compiled, statistical analysis performed and the interpretation of the results must be done.

COMMENTS AND RECOMMENDATIONS: Macrophytes can be sampled and analysed during a field survey along with other more routinely sampled benthic organisms used to monitor the environment, as aquatic insects and mollusks. Potential biomonitor macrophyte species in the Canadian environment would be *Eriocaulon septangulare*, *Eleocharis acicularis* and *Potamogeton richardsonii*. Then a comparison of the usefulness of macrophytes as biomonitor organism *vs.* the other more «standard» organisms can be done.

**MACROPHYTES**

METHOD NAME: Biochemical indicators of stress

PURPOSE AND DESCRIPTION: Biochemical responses to chemical effects are based on the principle that all toxic effects begin with a relation between the toxic chemical and some biochemical receptor in a living organism. Toxic effects on ecosystems begin with these chemical reactions in individuals. Detection and measurement of these chemical reactions in individuals should provide specific and sensitive diagnostic tools that give early warning, since the chemical reactions are unique and precede all effects at higher levels of organization, therefore before damage can be seen at a larger scale. Phytochelatins, resembling animal metallothioneins, and enzyme induction (particularly peroxidase activity) appear to be promising biomonitoring tools in areas contaminated with metals due to mine activities.

LIMITATIONS: Phytochelatins are not only synthesized when plants are subjected to environmental contamination; they can also be normally present in cell cytosol, playing a role in $Cu^{2+}$ and $Zn^{2+}$ homeostasis. Similarly, peroxidase is a normal constituent of the cell, and the enzyme activity can be induced not only due to metal exposure, but also to an array of other pollutants. However, the presence of external metal contamination is consistently related to high responses from these two biochemical indicators.

COSTS: - The field work consists of the collection of plant and sediment samples → see the previous method.

- Phytochelatins are not yet measured on a routine basis by private laboratories, but these would be done by institutional or govermental laboratories. In Canada, in the laboratory of Dr. Rauser, phytochelatins in plant samples are routinely measured using HPLC. His address is: Dr Wilfried E. Rauser, Department of Botany, University of Guelph, Guelph, Ontario, N1G 2W1.

Useful reference: Rauser 1990

- Enzyme activities (e.g. peroxidase) in plants can probably be determined in private laboratories.

Useful references: Van Assche and Clijsters 1990, Roy and Hänninen 1994.

-Concurrently with the measurements of phytochelatins and enzyme activities, the metal content of the plant must also be determined, as well as the free-metal ion concentrations of the sediment interstitial water.

COMMENTS AND RECOMMENDATIONS: Biochemical indicators of stress in plants appear as promising a biomonitoring tool as biochemical indicators already in use in animals. However, up to now, no field study related to metal contamination due to mine activities is reported in the literature.

**PHYTOPLANKTON**

METHOD NAME: Community size distribution

PURPOSE: This method attempts to monitor the effects of metals on the algal community by measuring changes in its size structure. The premise of this method is that, in the presence of metal pollution, there is a shift towards dominance of small organisms. This hypothesis has been confirmed in several experimental studies and agrees with some field observations.

DESCRIPTION: This method is based on the enumeration and measurement of algae in a phytoplankton sample. This can be achieved with a microscope equipped with a computer-assisted image analyzer. Alternatively, automated counting could be done with a Coulter Counter. The algae are grouped in logarithmic size classes. Because there is no need for taxonomic classification, operator training is minimal.

LIMITATIONS: Because the method has not yet been tested in field situations, it is not possible to know the detection limits. Responses to other stresses, like acidification and eutrophication, can lead to opposite trends. Interpretation could be difficult in the presence of multiple stresses. As for all the other methods, it is necessary to repeat the measurements during the year and to test the impact site against several reference sites. Only in this way, it is possible to distinguish the pollution effects from changes related to temporal and spatial variation.

COSTS: Sampling: small boat to reach the center of the lake. Van Dorn bottle or plastic tube to collect an integrated sample of water.

Sample preparation: fixing water samples with Lugol's solution (~$20.00 for fixing 100 samples).

Instrument: Inverted microscope equipped with a computer assisted image analyzer or Coulter Counter.

Operator: Experience with microscope but taxonomic training not necessary. If Coulter Counter, experience with the use of this instrument.

Time per sample: 1-2 hours for microscope; <10 minutes for Coulter Counter.

COMMENTS AND RECOMMENDATIONS: This method is easy and fast but it still needs field testing.

**PHYTOPLANKTON**

METHOD NAME: Diatom deformities

PURPOSE: This method uses deformities of diatom siliceous frustules to infer present or past (in sediment cores) pollution. It is based on the observations of increased percentages of anomalous frustules in severely contaminated environments.

DESCRIPTION: Most deformities compose asymmetrical development of the valves. These observations can be easily done with a normal microscope on slides of diatom frustules cleaned by acids or peroxide. More subtle abnormalities have been observed with an SEM microscope. Percentages of deformed frustules are measured in the impacted sites and in some reference sites since a low percentage of deformities can also be observed in uncontaminated sites.

LIMITATIONS: It is not yet clear if this phenomenon is widespread. In many studies of impacted sites, abnormalities are not mentioned, possibly because they are absent or overlooked by the operator. In some lakes, planktonic diatoms become very rare under severe pollution, so it would be easier to detect these abnormalities in the sediments where the diatoms accumulate.

COSTS: Sampling: collection of shallow sediment cores from a boat.

Sample preparation: digestion of sediment samples with $H_2O_2$. Diatom mounting with highly refractive resin (e.g. Naphrax). Cost of chemicals: ~$200.00 for 500 samples.

Instrument: microscope.

Operator: experience with microscope; some knowledge of diatoms but identification not necessary.

Time per sample: 3-4 hours sample preparation (many samples, up to 20 to 30, can be prepared together). One hour of microscope analysis.

COMMENTS AND RECOMMENDATIONS: If sediments are collected for metal analysis, it would be easy and rather economical to examine a fraction for presence of diatom deformations.

**PHYTOPLANKTON**

METHOD NAME: Canonical correspondance analysis of species composition

PURPOSE: The taxonomic composition of impacted sites can be altered compared to reference sites since tolerant species replace sensitive ones. These shifts in composition can be used to monitor the effects of metal pollution.

DESCRIPTION: Water samples integrated over the epilimnion are fixed in Lugol's. Algae are enumerated and classified using an inverted microscope.

LIMITATIONS: This method require a calibration set based on a large number of lakes usually at least 30-40. The classification requires a highly trained operator.

COSTS: Sampling: small boat to reach the center of the lake. Van Dorn bottle or plastic tube to collect an integrated sample of water.
Sample preparation: fixing water samples with Lugol’s solution (~$20.00 for fixing 100 samples).
Instrument: Inverted microscope.
Operator: Highly trained in algal taxonomy.
Time per sample: 3-4 hours.

COMMENTS AND RECOMMENDATIONS: Of the methods based on the species composition of phytoplankton this is the most promising but also the one requiring more effort, at least at the beginning, to establish a calibration set.

**PHYTOPLANKTON**

METHOD NAME: Pigment composition

PURPOSE: Algal pigments are differently affected by metals. Differences in the ratio of carotenoids/chlorophylls or pheophytins/chlorophylls could be indicators of metal induced stress in the community.

DESCRIPTION: Water samples are filtered and the filters extracted with ethanol or acetone. The extracts are read at various wavelengths with a spectrophtometer or analyzed by HPLC chromatography. Pigment analysis is the standard method for fast measurements of algal biomass. Only recently the analysis of the full spectrum by HPLC has been exploited to get information on the algal composition.

LIMITATIONS: This method is still tentative. The ability of changes in pigment composition to monitor metal related stress has to be tested in mining situations.

COSTS: Sampling: small boat to reach the center of the lake. Van Dorn bottle or plastic tube to collect an integrated sample of water.

Sampling preparation: Filtering samples on Glass fiber filters (Whatman GF/C or Gelman Type A/E; ~$50.00 for 100 filters). Storage in freezer. Extraction with ethanol, methanol, or acetone.

Instrument: Spectrophotometer or High Pressure Liquid Chromatography (HPLC) instrument.

Operator: Unskilled for spectrophotometry. Familiarity with the instrument for HPLC.

Time per sample: 10 minutes for spectrophotometry (reading at different wavelengths); 30 minutes for HPLC.

COMMENTS AND RECOMMENDATIONS: This method has the potential to be a useful monitor of metal stress. Analyses are fast and easy so that many replicate samples can be collected and processed in short time. Before its routine application, a complete test of the methods is imperative.

## PHYTOPLANKTON

METHOD NAME: Analysis of phytochelatins

PURPOSE: Like other plants, algae respond to elevated, potentially toxic, metal concentrations by producing phytochelatins. These peptides detoxify intracellular metals by binding them. This method utilizes the induction of phytochelatins as an indicator of metal stress in the environment.

DESCRIPTION: Phytochelatins dosage can be done using HPLC chromatography. The preparation of samples for chromatography involves several steps. The very sensitive technique has been described only recently and it is not yet routinely used.

LIMITATIONS: This technique so far has been applied only to marine algae. However, in theory, it should be applicable to freshwater. It appears most promising for monitoring Zn, Cu, and especially Cd, since these metals more readily induce phytochelatin production.

COSTS: Sampling: small boat to reach the center of the lake. Van Dorn bottle or plastic tube to collect an integrated sample of water.

Sampling preparation: filtering samples on Glass fiber filters (Whatman GF/C or Gelman type A/E; ~$50.00 for 100 filters). Storage in liquid nitrogen. Filter extraction involves various steps: acid treatment, grinding, centrifugation, addition of a fluorescent tag, reaction with several compounds.

Instrument: HPLC.

Operator: Good experience with chemistry and use of HPLC. Because method still tentative, ability to experiment with new technique.

Time per sample: 4-6 hours for preparation of samples (several samples can be prepared together). Thirty (30) minutes for HPLC when analysis become routine, but time needed to test the technique.

COMMENTS AND RECOMMENDATIONS: This method is still to be developed for monitoring. In the only application to a natural phytoplankton population, phytochelatins were detected before any effects on algal growth was apparent. The method has the potential to become an early warning indicator of metal contamination.

**PHYTOPLANKTON**

METHOD NAME: Pollution Induced Community Tolerance

PURPOSE: In this method, the capacity of plankton communities subjected to elevated metal concentrations to increase their tolerance is exploited to detect contamination exposure.

DESCRIPTION: Samples from the test site are incubated with increasing concentrations of the metals suspected to contaminate the lake. Algal production under a concentration series of test substances is monitored by $^{14}C$ uptake and compared with the response of reference samples from uncontaminated environments. Exposure to metals is suspected if the community production is less inhibited than that of references.This method does not require a highly trained operator and the equipment requirement is minimal.

LIMITATIONS: Because cotolerance between various metals can develop, the response may not be specific. For example, a community could show tolerance to copper even if it was exposed to cadmium or zinc. The base-line tolerance for a reference community is not always easily established. There could be large variations related to season and trophic status.

COSTS: Sampling: small boat to reach the center of the lake. Van Dorn bottle or plastic tube to collect an integrated sample of water.

Sampling preparation: algal incubation in bottles with addition of $^{14}C$. Filtering of samples after incubation on Millipore or Nucleopore filters (~$100.00 for 100 filters).

Instrument: Incubator, Geiger or Scintillation Counter for measuring $^{14}C$ incorporation.

Operator: Some experience working with isotopes.

Time per sample: 3-4 hours incubation, plus 2 hours for filter preparation and radioactivity reading. NB: up to 20 samples can be prepared together.

COMMENTS AND RECOMMENDATIONS: So far, this method has been applied only in Sweden. It should be more specific than methods based on species composition which can be altered by other factors, anthropogenic and not, than metals.

**PERIPHYTON**

METHOD NAME: Niche center gradient analysis

PURPOSE: Under field conditions, several different diatom species shown different patterns of abundance with varying concentrations of metals. This method calculates a niche center index to indicate the relationship of a diatom species to an environmental gradient of metals.

DESCRIPTION: The niche center index locates the effective mean position of the population distribution of a single taxon along the weighted environmental gradient. The calculation is done by summing diatom populations in each class on the environmental gradients, multiplying by the weight factor for that class and by a number representing the position of the class on the gradient. This product is then summed over classes and divided by the number of classes to give the average position of the species on the gradient. Species were selected according to their occurrence at the high or low ends of the environmental gradients.

LIMITATIONS: This method requires some efforts for validation. Diatoms are not necessarily the most abundant or ecologically important group of algae.

| COST: Depending of technician or professional experiences: | / sample |
|---|---|
| Because taxonomic identification and counts are limited to diatoms, efforts are minimum (1 to 2 hours): | ~$50.00 to $100.00 |
| Sampling: | ~$100.00 to $150.00 |

| | |
|---|---|
| TOTAL: | ~$150.00 to $250.00 |
| Sampler/station (one time): | ~$150.00 to $200.00 |
| Calibration set: | ~$4,000 to $10,000 |

COMMENTS & RECOMMENDATIONS: Methods based on the diatom species composition have demonstrated their applicability. The interest of the method rests in their used to study environmental condition changes *vs*. time. However, the use of this method requires some efforts of validation and at the beginning of a study can necessitate a calibration set.

**PERIPHYTON**

METHOD NAME: Dissimilarity index, community diversity and species evenness

PURPOSE: The taxonomic composition of contaminated sites *vs*. reference sites differs by the dominance of species of a corresponding tolerance with simultaneous absence of all less tolerant forms. This changes in taxonomic composition can be used to monitor the presence of metal contamination.

DESCRIPTION: Substrata is taken and scraped in sterilized water before being fixed in Lugol's. Algae are identified and enumerated using an inverted microscope. These indices are calculated from the abundance, in assemblages, of each taxon or of individuals in each species.

LIMITATIONS: This method requires specialized expertise for identification. These indices necessitate identification of reference sites not different from contaminated sites other than for contaminants analyzed.

| COST: Depending of technician or professional experiences: | / sample |
|---|---|
| Taxonomic identification and count (2 to 4 hours): | ~$100.00 to $200.00 |
| Sampling: | ~$100.00 to $150.00 |

| | |
|---|---|
| TOTAL: | ~$200.00 to $400.00 |
| <u>Sampler (one time):</u> | <u>~$150.00 to $200.00</u> |

COMMENTS & RECOMMENDATIONS: Each index can be effectively coupled to other mathematical indices to compare periphytic microalgal structure between contaminated and reference sites. These indices are usually used in studies of metal impacts on periphyton.

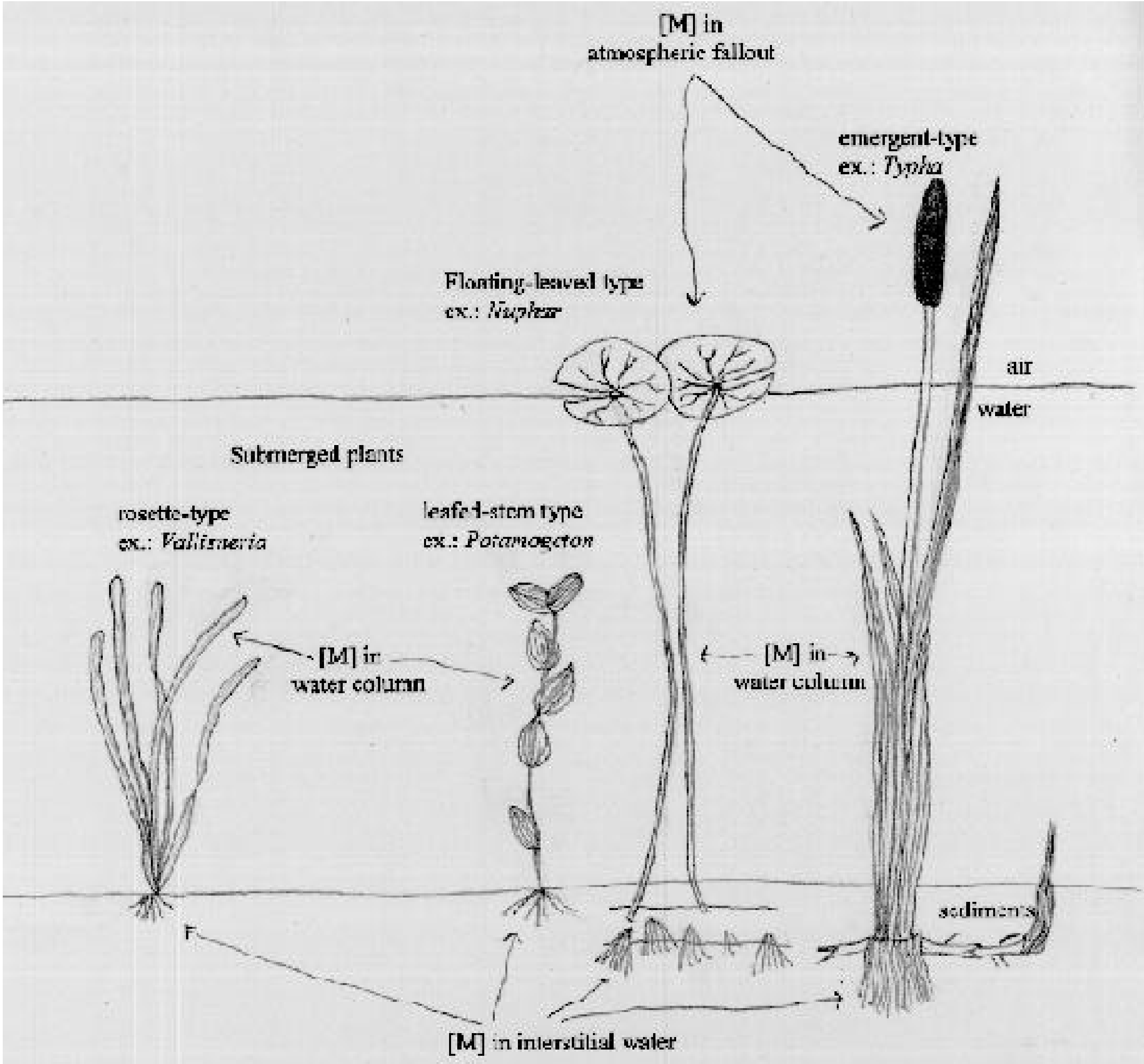


Figure 1: Schematic representation of various types of aquatic plants and their possible pathways for metal [M] uptake.