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Accumulation of Copper in Freshwater Algae

Introduction

Copper is an essential micronutrient for living organisms. As one might expect, normal cells have mechanisms for taking in copper, and they contain copper, although not necessarily in concentrations high enough for detection. The functions and the mode of entry of copper in cells, and the toxic effects of copper, are discussed elsewhere in numerous publications.

For aquatic biota, sources of copper include the water (including dissolved and suspended material), the sediment, and the nutrient materials, or any combination of these. Because of its tendency to combine with a wide variety of organic molecules, copper, not surprisingly, binds to sites within and upon living cells. These include sensitive and insensitive sites; indeed, the diversion of the metal to sites insensitive in terms of toxicity has potential for protecting cells against the toxic action of copper (Passow et al., 1961).

Certain plants and animals, or certain tissues or organs, accumulate abnormally high levels of copper. Such accumulation can be attributed to high levels of copper in the substrate or medium in which the organism lives, or to unusually metabolic activity, or to both. Accumulation of extremely high levels may be accompanied by pathological symptoms, but this is frequently not so. The more general tendency for aquatic organisms to contain higher concentrations of copper than their surroundings is illustrated in the values given by Bowen (1966); for example, for marine systems, typical values for plants are 11 ppm, for animals 4 to 50 ppm, and for water 0.003 ppm. The value for copper in fresh water is 0.01 ppm; and although no corresponding values are given for aquatic biota, those from recent literature range from 1.0 to 1000 ppm for plants and from 0.1 to 100 ppm for fish, with much higher values than these for situations of severe copper pollution.

This concentration phenomenon has been quantitatively expressed by the use of a *concentration factor*, defined by Polikarpou (1966) as the ratio of the concentration of a substance in biological material (expressed in ppm dry weight) to its concentration in water (in ppm) or in the previous trophic level of the food chain (same units). The ratio is normally based on total levels of a substance without distinction as to chemical form, and Polikarpou has emphasised the need for steady-state of equilibrium conditions when determining concentration factors.

Although it is reasonable to determine concentration factors for organisms or cells in defined experimental systems, in field situations, and in complex experimental systems, caution has to be observed in the use of such determinations. For plants in aquatic systems the concentration factor is expressed on the basis of the concentration in water; it can also be expressed in terms of sediment concentration. For heterotrophs there are clearly multiple sources; for example, bivalves in a Hudson River aquatic food chain are exposed to at least four sources of heavy metal: the water, the plankton, the detritus, and the bottom sediment (Kneip and Lauer, 1973). A simple single-source concentration factor could thus be misleading. When models are in use, it may be possible to compute a single concentration factor for all sources based on the transfer coefficients for the individual sources. More frequently, however, a separate concentration factor is calculated for each source, and only the value for the water source is present.

From an overview of existing information on the accumulation and biotransformation of copper in freshwater biota, there emerges a clear impression of the need for an integrated multidisciplinary approach to the subject. Valuable but as yet disconnected bodies of appropriate information are available within the disciplines of biochemistry, pollution monitoring, applied ecology, toxicology, plant physiology, animal physiology, and analytical chemistry. For example, much of the information on biochemical and biophysical aspects of copper toxicology and intracellular binding comes from studies on mammalian cells and not from studies of aquatic biota.

Although there are fundamental similarities between the cells of plants and lower animals and those of mammals, at the level of organismal and tissue organization there exist basic difference which should caution against the making of broad generalities or against extrapolation.

Another problem is that many of the available field data on copper in aquatic biota come from investigations of heavily polluted streams and lakes, which rarely involve a single pollutant metal. Control or reference material has to be taken from very carefully selected sites, and these are rarely controls in the purest sense. The need for monitoring pollutants in aquatic systems have provided incentives for extensive examination of metal accumulation in aquatic biota; but because copper is only moderately toxic to mammals and does not accumulate to very high levels in freshwater fish which human beings consume, copper in freshwater biota has received less attention than, for example, mercury or lead in fresh- and saltwater biota. Nevertheless, the sensitivity of algae to copper has long been recognized and has apparently stimulated interest in the physiological effects of copper on freshwater plants, especially algae, and a number of laboratory studies have been carried out in this area, with varying attempts to relate results to field situations. To date, studies on the effects of copper on fish have been concerned more with toxicity than with accumulation and physiology.

Factors Affecting Bio-concentration of Copper

The similarity of concentration factors from water of different chemical composition and copper content suggests that the amount of copper taken up is a function of the concentration in the water. This has been confirmed experimentally by studies on a number of species, including *Chlorella pyrenoidosa*, at 0.015 to 1.0 ppm Cu (Knauss and Porter, 1954); *Scenedesmus acutiformis*, at 0.1 to 0.5 ppm Cu (Stokes et al., 1973); *Chlorella vulgaris*, at 0.05 to 0.75 ppm Cu (Foster, 1977); and *Chlorella regularis*, at 0.22 to 2.0 ppm Cu (Sakaguchi et al., 1977). Comparisons between studies are often difficult to make because of the effects of other factors, including pH.

Steeman-Nielsen et al. (1969) showed that *C. pyrenoidosa* cells exposed to 0.05 ppm Cu for 2 hours at pH 5.0 removed 6 to 7% of the copper, compared with 54 to 80% at pH 8.0. Cells of *S. acuminatus* exposed to 0.1 ppm Cu for 20 minutes contained 400 µg/g at pH 4.8, 750 µg/g at pH 5.8, and 4000 µg/g at pH 6.8 (Mierle and Stokes, 1976). This study followed a time course and showed that the pattern of uptake at pH 6.8 and additional slower, continued uptake was observed and interpreted as intracellular uptake.

The presence or absence of oxygen can have a profound effect on copper uptake by algal cells. McBrien and Hassal (1956) showed that the cells of *C. vulgaris* bound more copper under anaerobic conditions. Thus it is important in experimental procedure to avoid conditions that inadvertently cause the development of anoxia (e.g., unstirred cultures, pelleted algal cells) and to specify field conditions such as the development of anoxia in metal-polluted lakes. Preliminary results indicate that copper accumulated under anoxic conditions is not irreversibly bound, and that cells containing rather high concentrations of copper are viable and will recover (Mierle and Stokes, 1976).

The presence of other ions, especially divalent cations, has been shown to affect copper binding by algal cells. It is well known that copper toxicity is ameliorated in hard water, and recent studies have confirmed that calcium ions inhibit the uptake of copper. Mierle and Stokes (1976) showed that 0.4 ppm Ca decreased the 2 hour concentration factor of copper by *S. acuminatus* from 10,000 to 1000, and Sakaguchi et al. (1977) reported that the copper content of *C. regularis* exposed to 2.0 ppm Cu at pH 4.6 for 65 minutes with 1 meq Ca was 2000 ppm, compared with 4000 ppm in the absence of calcium.

Organic chelators, present in natural water and frequently added to culture solutions, not only decrease toxicity but also affect uptake of copper. Sunda and Guillard's (1976) model for the marine diatom *Thalassiosira pseudomonada* relates copper content of cells to cupric ion activity and not to total copper concentration. More indirectly, similar evidence exists for uptake of ionic copper by freshwater algae.

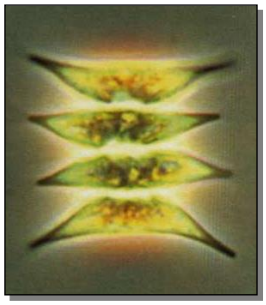
Thus Manahan and Smith (1973), working on micronutrient requirements for *C. vulgaris* and *Oocystis marssonii*, demonstrated that the growth response to copper in the non-toxic range (up to 0.03 and 0.04 ppm) could be controlled either by decreasing copper concentration in the absence of EDTA, or by maintaining total copper constant and increasing EDTA. Many studies of copper toxicity to algae have indicated that organically bound copper is not toxic, and Stokes (1975) has shown that both toxicity to and uptake of copper by *Scenedesmus* can be decreased by additions of EDTA to the medium.

Table 1. Concentration Factors for Algae in Fresh Water.

Organism	Source of Material	Concentration Factor[a]	Reference	Additional Comments
Cladophora glomerate	Lake Ontario (urban area)	2.2×10^3	Keeney et al. (1976)	Field collection
C. glomerata	Lake Ontario (remote area)	1.9×11^3	Keeney et al. (1976)	Field collection
C. glomerata	Lake Erie (eutrophic)	1.0×10^3	Taft and Kishler (1973)	Field collection
C. glomerata	Spokane River (industrial pollution)	2.5×10^3	Funk (1973)	Field collection
Cladophora sp.	Lower Swansea Valley (distant from zinc smelter)	1.8×10^3	Trollope and Evans (1976)	Field Algal Bloom
Cladophora sp.	Lower Swansea Valley (distant from zinc smelter)	3.5×10^3	Trollope and Evans (1976)	Field Algal Bloom
Tribonema sp. (a)	Lower Swansea Valley (adjacent to zinc smelter)	1.3×10^4	Trollope and Evans (1976)	Field Algal Bloom
Tribonema sp. (d)	Lower Swansea Valley (adjacent to zinc smelting waste)	8.3×10^4	Trollope and Evans (1976)	Field Algal Bloom
Microspora	Lower Swansea Valley (near zinc smelting waste)	1.6×10^4	Trollope and Evans (1976)	Field Algal Bloom
Chlorella regularis	Laboratory experiment	1.5×10^3	Sakaguchi et al. (1977)	Experimental pH was 4.6; very high Cu levels, 0.5-2.0 ppm supplied
Scenedesmus acutiformis	Laboratory experiment, batch culture	$0.87\text{-}1.5 \times 10^4$	Stokes (unpublished data)	8 days' growth; copper-tolerant alga
S. acuminatus	Laboratory experiment, continuous culture	4.0×10^3	Mierle and Stokes (1976)	Short-term uptake, 20 min exposure
Chlorella vulgaris	Laboratory experiment	$0.7\text{-}1.0 \times 10^3$	Foster (1977)	Copper-tolerant alga
C. vulgaris	Laboratory experiment	$0.4\text{-}4.6 \times 10^3$	Foster (1977)	Non-copper tolerant alga

[a]Where necessary, concentration factor has been calculated from published values or values approximated from graphs.

Scenedesmus



Cladophora

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