

Chapter 18

EFFECTS OF HEAVY METALS ON THE AQUATIC BIOTA

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INTRODUCTION

This review focuses on the effects of heavy metals on the aquatic (primarily freshwater) biota. During the 1970s and 1980s there was intense research interest in the biological effects of heavy metals. While this has provided a firm foundation for the interpretation of toxic effects associated with metalliferous mining in particular, many other aspects of mining, with potentially adverse effects on the biota, have received less attention. In particular, physical factors, such as those associated with high suspended solid loads, for example, are studied comparatively rarely, although for many deposits of insoluble metals (e.g., gold, tin) such factors may be more significant than toxicity (LaPerriere et al., 1985; Van Nieuwenhuysse and LaPerriere, 1986; Briones, 1987). Moreover, discovery of a valuable mineral may lead to large increases in population. The town of Jos in Nigeria, for example, owes its existence to the tin mines in the region and the main water quality problem in the Delimi river downstream of the town is due to sewage pollution rather than to mining (Kelly and Ali, 1993). Further environmental impacts can include clearing land for agriculture to feed the growing population (Livett et al., 1979). It would be wrong to consider the potential impact of a new development without considering these aspects as well.

Toxic effects of mining wastes, however, remain the prime subject of concern, particularly because of potential impacts on human health due to the movement of heavy metals through food-webs and into organisms consumed by human populations. The ecological impact depends upon the particular metal, or cocktail of metals, in question. This can be further modified by other factors related to the background geology (acidity, water hardness, etc.) which affect the speciation of the metal. The biological environment can also affect toxicity: of particular note is the methylation of some metals (particularly Hg) under anaerobic conditions to form extremely toxic compounds (Moore and Ramamoorthy, 1984). Bioaccumulation of methylated Hg was responsible for the outbreaks of "Minamata disease" in areas of Japan, where humans consumed diets rich in Hg-contaminated fish (Clark, 1986; Honma, 1988).

The term "heavy metal" is traditionally reserved for metals with a density >5 (e.g., Passow et al., 1961) although lighter metals (e.g., Al) have often been included as well. However, toxic effects within this group are not constant and attempts have been made to abandon the term heavy metal in favor of a more biologically-relevant classification (Nieboer and Richardson, 1980). In

this classification, metal ions are grouped into three groups based upon their affinity for different ligands. Class A metals (e.g., K, Ca, Ba) tend to seek oxygen-containing ligands while Class B metals (e.g., Cu(I), Ag, Au, Hg) seek nitrogen- and sulphur-containing ligands; Borderline metals (e.g., Ni, Cu(II), Zn, Cd, Pb) with intermediate properties are grouped with the Class B metals, as they have similar toxic effects. Although unsuccessful in displacing the term "heavy metal" from the public imagination, this classification does provide a set of criteria by which metals may be compared toxicologically.

The purpose of this review is to draw generalizations about the toxic effects on the aquatic biota of metals associated with mining activities. For a detailed review of this sizeable and complex subject, the reader is referred to general references such as Kelly (1988) and references contained therein.

UNDERLYING ECOLOGICAL CONCEPTS

Central to an understanding of the ecological effects of pollution is the concept of "stress," defined as "external constraints limiting the rate of resource acquisition, growth or reproduction of organisms" (Grime, 1989) or "an environmental condition that, when first applied, impairs Darwinian fitness" (Sibley and Calow, 1989). These definitions apply principally to individual organisms; however, the integrated effects on individuals may ultimately affect community structure. Although the concepts of succession (the natural sequence of changes in an ecosystem over time), and climax (the equilibrium community at the end of a succession) are difficult to apply in flowing waters, biologists recognize, implicitly or explicitly, an "expected" or "ideal" community that should be present for a given river type. Departures from this suggest that some form of external stress is acting on the system, although it is important to recognize that many forms of stress are natural (i.e., extremes of temperature, tidal movements, etc.) and, indeed, stress is the driving force behind natural selection and evolution (Hoffman and Parsons, 1993).

Early studies on the ecological effects of heavy metals noted changes in distributions of plants and animals downstream of mines. Implicit were the assumptions that the mines were responsible for some degree of ecological perturbation, and that the expected range of species present at those sites had changed in direct response to the mining. For example, Carpenter (1924, 1926) noted an impoverished fauna immediately downstream of a

Pb mine in Mid Wales that she attributed to the toxic effect of Pb in the water, although it is now considered that the observed effects were more likely to have been caused by Zn. When mining ceased, the fauna recovered from 14 species in 1920 to 130 species in 1949 (Fig. 18.1; Jones, 1949) and appears to have remained at this state subsequently. Recovery, therefore, is a relatively slow process limited, perhaps, by the rate at which individuals from new species are able to immigrate to the river, although evidence for this is largely circumstantial.

The "struggle for existence," first described by Darwin (1859), occurs primarily within populations of the same species, rather than between communities of many different species. For any particular population exposed to heavy metal toxicity downstream of a mine, some individuals may prove more tolerant than others and these will survive and, possibly thrive in the absence of competition. It is a classic case of natural selection: successful (i.e., tolerant) individuals pass their genotypes onto the next generation which therefore contains a greater proportion of the genes responsible for tolerance. Over time, populations come to be composed almost entirely of tolerant individuals. This has been shown particularly clearly in the algae (see below) and some grasses (Bradshaw and McNeilly, 1981).

However, particular species within a community will also vary in the extent to which they are affected by a toxin. This may be due partly to physiological differences between species but also to ecological or behavioral factors: plants may vary in the proportion of their surface area that is exposed to the water, while invertebrates that ingest large quantities of sediment may accumulate higher concentrations of toxin than species that ingest leaf material. Thus, the interrelationships between organisms in a stream will change. A species that is particularly sensitive may decline in abundance while one that is more tolerant will increase. As the

concentration of the toxin increases, so some species may disappear altogether while at very high concentrations, conditions may approach those of biologically "extreme" environments such as saline lakes and thermal springs. However, while these "extreme" environments tend to be dominated by microorganisms (Brock, 1969), all groups of photosynthetic taxa of any quantitative importance in freshwaters have a few representatives that are tolerant of elevated metals (Whitton, 1980). When the level of environmental stress is particularly high, then it becomes the principal determinant of species numbers at a site. At lower stress levels, however, other factors (e.g., diversity of habitats, nutrients) may also affect species numbers (Fryer, 1980). This was particularly clearly illustrated for heavy metals in a study by Whitton and Diaz (1980) who plotted the number of photosynthetic organisms found in 10-m lengths (termed "reaches") of an enormous number of sites in the U.K., Europe, and the United States against the concentration of Zn in the water (Fig. 18.2). The number of taxa at sites with low Zn concentrations is highly variable, but at sites with high Zn concentrations it is always low.

The reduction in competition from less tolerant individuals at high concentrations of heavy metals may enable the few species that are tolerant to thrive. Thus, mine adits are often characterized by lush growths of algae (Armitage, 1979; Patterson and Whitton, 1981) possibly due to a lack of competing algae (Leland and Carter, 1984) or invertebrate grazers (Klotz, 1981). The massive growths themselves can change conditions within the stream, either by smothering the substrate (Armitage, 1979) or causing deoxygenation of the water at night, so interpretation of these effects is not necessarily straightforward.

The relationship between primary producers and grazers introduces a further ecological concept necessary to understand the effects of heavy metals on the biota. This is "bioaccumulation," derived from the fundamental ecological idea of biogeochemical cycles in which the environment and different trophic levels (feeding strata in a food chain) are represented stochastically as compartments, between which elements are transferred over time. Organisms have well-developed mechanisms for absorbing, metabolizing and excreting nutrients such as nitrogen and phosphorus. Indeed, they can even absorb, metabolize, and excrete metals at concentrations that are encountered naturally. These mecha-

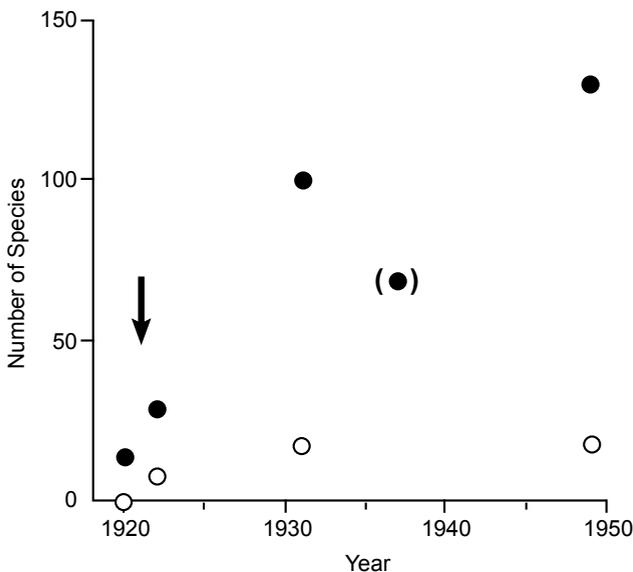


FIGURE 18.1—The recovery of the invertebrate fauna in the River Rheidol, Mid Wales, following the cessation of lead mining (arrowed). Closed circles = all invertebrates; open circles = Trichoptera species only; bracketed circle = sample collected after period of heavy rain. Compiled from published sources by Kelly (1988).

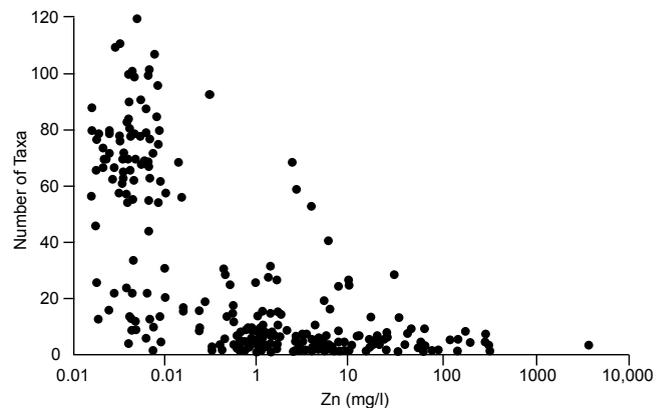


FIGURE 18.2—Relationship between Zn in water and number of species in 10-m lengths of streams and rivers in U.K., Western Europe and U.S.A. Adapted from Whitton and Diaz (1980).

nisms, however, can become saturated at high concentrations of metals, and the rate of uptake can exceed the rate of loss, a more energetically demanding process. For this reason, organisms can accumulate high concentrations of heavy metals in their tissues. During its lifetime a predator will consume many times its own body weight of prey and if this prey has high concentrations of metals, then the predator, similarly unable to metabolize and excrete the metals, will accumulate yet higher concentrations in its tissues. This idea of "biomagnification" along a food chain was famously studied for pesticides (Carson, 1963) and is relevant to studies of heavy metals as well (Anderson, 1977), although the evidence is often equivocal (Kelly, 1988 and see below).

ACCUMULATION OF HEAVY METALS

It has been understood for about thirty years that organisms can accumulate heavy metals to much greater concentrations than those found in their environment. "Enrichment ratios" (concentration in organism/concentration in water, also known as "concentration factors") are typically in the range 10^3 – 10^4 , although these tend to decrease as the aqueous concentration increases (Kelly, 1988). However, concentrations within organisms at any site can vary considerably, depending upon growth strategies (in plants), feeding strategies (in animals) and basic physiological differences. In general, there is a good relationship between the aqueous concentration of a metal and its concentration found in plants (Fig. 18.3). Deviations from this trend can be partly explained by environmental factors such as low pH, which limits metal accu-

mulation, and the use of metal exclusion by some algae as a tolerance mechanism (Kelly, 1988).

Similar relationships can be established for individual species; a number of plants have been studied in this way (Wehr and Whitton, 1983; Whitton et al., 1989; Kelly and Whitton, 1989a and b). A comparison of metal concentrations in three algae and four bryophytes as a function of metal concentrations in water (Fig. 18.4) indicated that the bryophytes accumulated more Zn, Cd and Pb than the algae, although the green algae (*Cladophora glomerata*, *Stigeoclonium tenue*) had steeper slopes, indicating a greater change in accumulated concentrations for the same change in aqueous concentrations (Kelly and Whitton, 1989a); the red alga *Lemanea* had a similar slope to the bryophytes but contained lower overall concentrations. In rooted vascular plants, metals also enter the plants via the sediments and, in general, higher concentrations are found in roots than shoots (Fig. 18.3). In *Potamogeton crispus* and *P. pectinatus*, Cu, an essential trace element, was translocated from the roots to the shoots while Pb, which is not essential, was retained in the roots (Welsh and Denny, 1979).

The sediments are also a major pathway for uptake of metals by animals, with species living in close proximity to sediments (e.g., burrowing mayflies, Ephemeridae and some Chironomidae) having higher concentrations. Filter feeders, detritivores, algal grazers, carnivores, and surface feeding species have progressively lower concentrations (Smock, 1983a). Metals in the gut may represent a major proportion of the total body load (Ellwood et al., 1976) and, as a result, many workers keep invertebrates alive but unfed for 2–4 days prior to analysis to allow purging of the gut

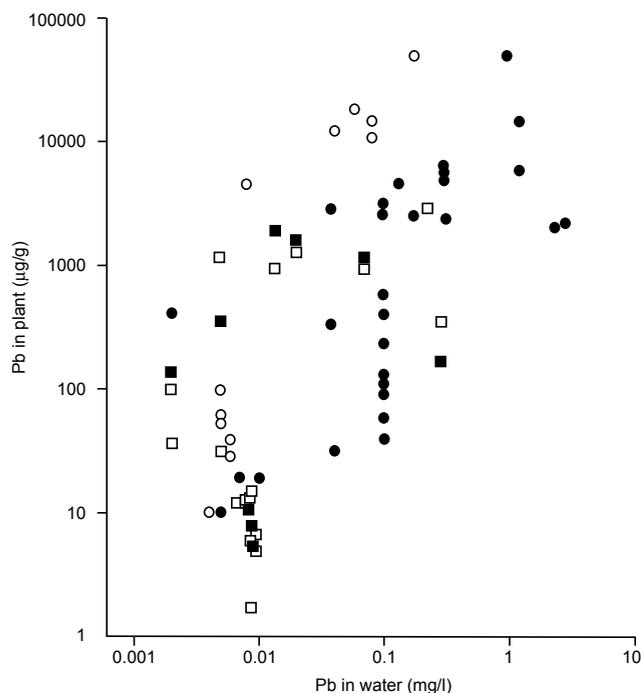


FIGURE 18.3—Accumulation of Pb from water by: algae (closed circles); bryophytes, or mosses (open circles); and angiosperms, or flowering plants (roots, closed squares; shoots, open squares). Compiled from published and unpublished sources by Kelly (1988).

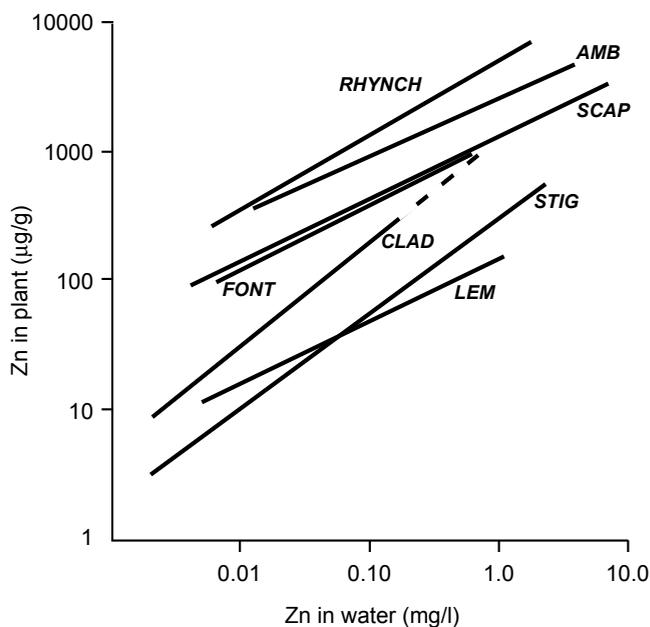


FIGURE 18.4—Influence of aqueous Zn on Zn concentration in plants. Algae: LEM, *Lemanea fluviatilis*; CLAD, *Cladophora glomerata*; STIG, *Stigeoclonium tenue*. Bryophytes: SCAP, *Scapania undulata*; AMB, *Amblystegium riparium*; FONT, *Fontinalis antipyretica*; RHYNCH, *Rhynchostegium riparioides*. After Kelly and Whitton (1989a).

contents. The unselective ingestion of sediment (which masks the biological “signal”) by many aquatic animals may be one reason why clear relationships between organismic and environmental concentrations of metals are harder to find in the literature compared with similar studies on plants. Other factors that complicate determination of metal uptake from sediments are direct accumulation from the water itself (Smock, 1983b; Rainbow and Moore, 1986) and the localization of metals within particular organs. Fish muscle tissue, in particular, often contains low concentrations compared to organs such as the liver and kidney (Murphy et al., 1978; Cowx, 1982; Bendell-Young et al., 1986). Thus, piscivorous (fish-eating) species such as perch (*Perca fluviatilis*) and pike (*Esox lucius*) have lower concentrations of Zn, Cd and Pb than bottom-feeding species such as roach (*Rutilus rutilus*) and bream (*Abramis brama*; Badsha and Goldspink, 1982) although at high metal concentrations, susceptible prey species may be eliminated and replaced by metal tolerant species. The effect on predators will depend upon whether tolerance is due to an ability to accumulate and detoxify a metal, or exclude it from cells. This will act either as a positive or negative feedback mechanism to enhance or weaken the transfer of metals through the food chain (Dallinger et al., 1987).

TOXICITY AND TOLERANCE

Toxicity of materials to aquatic populations has traditionally been expressed as “LC₅₀”: the concentration that would kill (“lethal concentration”) 50% of a batch of organisms within a specified time period (usually 48–96 h). Although this toxicity measurement is used widely for fish and invertebrates, unicellular algae present particular problems for the test, as a concentration that inhibits growth might not necessarily kill the cells outright.

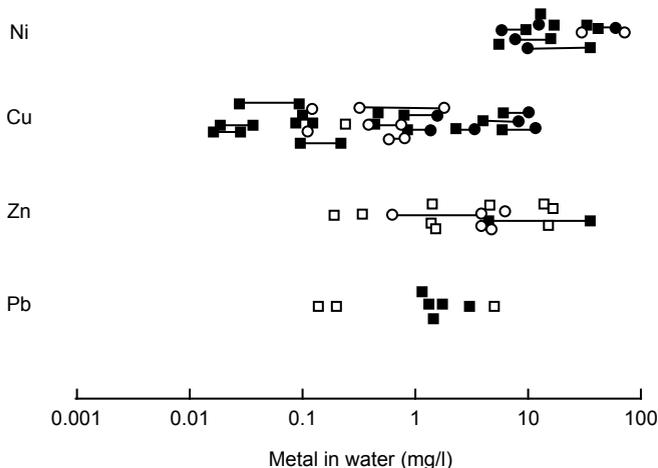


FIGURE 18.5—Effect of nickel, copper, zinc, and lead on freshwater fish, expressed as LC₅₀. No attempt is made to distinguish different taxa; however, distinctions have been drawn between 24-h LC₅₀ (closed circles), 48-h LC₅₀ (open circles), 96-h LC₅₀ (closed squares) and LC₅₀ values based on incubation times in excess of 10 days (open squares). Solid lines indicate range of effects obtained from different treatments. Compiled from published sources by Kelly (1988).

For this reason, the term LC₅₀ is often replaced by “EC₅₀”: the effective concentration required to reduce a particular parameter (usually growth) by 50%. In both cases, physical, chemical and biological aspects of the test can have major effects on results (Mance, 1987) although compilations of data are still useful for making comparisons between metals (Fig. 18.5) and between taxonomic groups (Fig. 18.6).

Although measurement of acute toxic effects is still valuable for some purposes, the emphasis in recent years, however, has moved to an understanding of the various components of environmental stress and their effects on ecological fitness (see above). Environmental effects on ecological fitness can take many forms. For example, juvenile life-stages are often more susceptible to heavy metals than adult stages (Watton and Hawkes, 1984; Nicola Giudici et al., 1987), reproduction may be impaired (Brungs et al., 1976; Sehgal and Saxena, 1986) and both invertebrates (McMurtry, 1984) and fish (Atchinson et al., 1987) show avoidance behavior when exposed to heavy metals.

To put this into context, the “Maximum Acceptable Toxicant Concentration,” assessed with reference to effects on reproduction and fecundity (fertility), for copper and lead was determined to be between two and three orders of magnitude lower than LC₅₀ values for these metals (Brungs et al., 1976). Moreover, molecular biological studies are now able to detect the “switching on” of genes coding for cellular systems involved in detoxification and metabolism of metals at even lower concentrations (Roch et al., 1986; George and Langston, 1994).

Plants

Many physiological processes have been shown to be disrupted by heavy metals and it is likely that there are cascades of toxic effects within cells. For example, many studies have shown photosynthesis to be reduced (Fångstrom, 1972; Brown and Rattigan, 1979; Rabe et al., 1982), possibly as the central Mg atom of the chlorophyll molecule is replaced by the heavy metal (Arndt, 1974). Other studies have shown respiration to be reduced (Bonaly et al., 1986) and photorespiration to increase (Filbrin and Hough, 1979). If such basic metabolic processes are damaged, then many

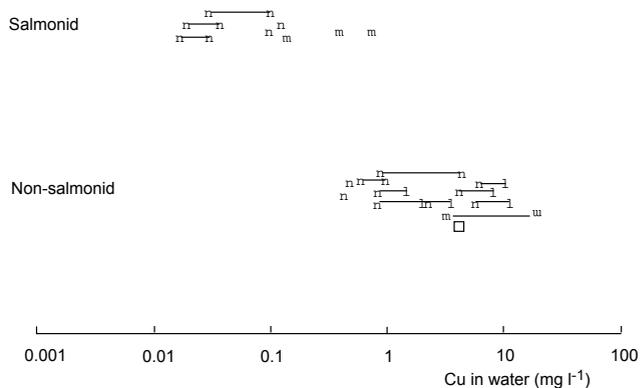


FIGURE 18.6—Comparative effects of copper on Salmonidae and other fish, expressed as LC₅₀. Details as for Figure 18.5. Compiled from published sources by Kelly (1988).

other cellular processes will also be affected, for example, breakdown of membrane integrity (Singh and Yadava, 1986) and inhibition of nutrient uptake (Kashyap and Gupta, 1982; Peterson and Healey, 1985). The result of these effects are illustrated by an unpublished study on *Chlorella* sp. (Fig. 18.7), which shows slower growth at higher Zn concentrations, leading to lower overall yields as cellular processes are disrupted by higher and higher concentrations of metal. At very high concentrations, cell numbers decline.

Tolerance can be induced in algae by sub-culturing in successively higher concentrations of metals (Stokes, 1975; Shehata and Whitton, 1982). As algal cultures tend to have very little genetic variability, it is assumed that such tolerance is due to the selection of spontaneous mutants. Tolerance has also been observed in field populations of green algae, where there tends to be a clear relationship with aqueous metal concentrations along with morphological changes (Harding and Whitton, 1976; Say et al., 1977).

Given the ease with which tolerance can be induced in the laboratory in a range of organisms, it seems likely that selective pressures will work simultaneously on many cellular processes, leading to many possible "solutions" to the problem of toxicity. Thus, Foster (1977) observed Cu-tolerant varieties of *Chlorella* sp. in the R. Hayle, Cornwall, that appeared to exclude Cu from the cell, whereas tolerant strains of *Stigeoclonium tenue*, another green alga, appear to accumulate much higher concentrations than non-tolerant ones (Kelly and Whitton, 1989b). Other common tolerance mechanisms include the production of extracellular products to "bind" metals into complexes (Clarke et al., 1987) and the production of metallothioneins (see below). The former have been studied particularly in the Cyanobacteria, where they are thought to be produced naturally in response to Fe starvation, but to have a secondary role protecting against elevated metal concentrations.

Animals

The literature on the physiological effects of heavy metals on animals is enormous and, at times contradictory, although the consensus seems to be that hypoxia (a deficiency in the amount of oxygen reaching bodily tissues) is the prime manifestation of

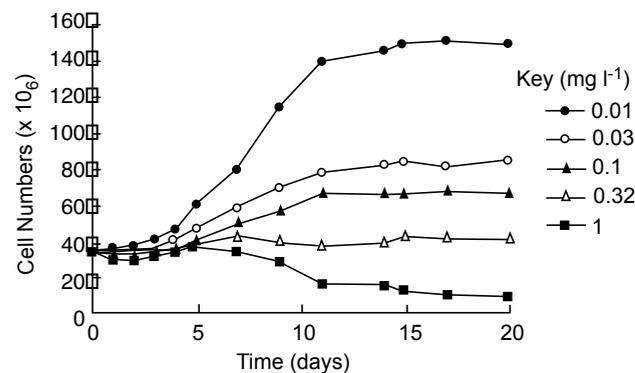


FIGURE 18.7—The effect of Zn on growth of *Chlorella* sp. Unpublished data of S.J. Oluwatoyin and M.G. Kelly. Rate of growth and yield are both negatively correlated with \log_{10} Zn concentration, whilst the length of the lag phase (the period between inoculation and start of exponential phase) is positively correlated.

metal toxicity (Burton et al., 1972). This is caused by a breakdown in gas exchange either from coagulation and precipitation of mucus or from cytological (cell) damage. Hypoxia is manifested in many ways, including increased heart rate, ventilation and coughing (Hughes and Tort, 1985), anemia (Tewari et al., 1987; Hughes, 1988) and oxygen dissociation (Hughes, 1988). The latter two may be related to changes in the hemoglobin molecule itself (due to displacement of the central Fe atoms), or changes in the synthesis of the hemoglobin (Jackim, 1973). Effects on respiration have also been observed in invertebrates (Hubschman, 1967; Correa, 1987). One consequence of these effects is a steady metabolic cost from exposure to sub-lethal concentrations of metals (Collvin, 1985). This becomes more pronounced when the organism has been starved prior to exposure (Segner, 1987) although a certain amount of acclimation has been noted when organisms are pre-exposed to the metal in question (Laurén and McDonald, 1987). The longer lifecycles of fish and benthic invertebrates, when compared with the microscopic algae discussed above, means that the development of tolerance over several generations has not been studied in the same detail. There is, however, a considerable literature on the role of metallothioneins (see below) in fish.

Effects on behavior and reproduction were mentioned above. One particularly bizarre cause of reproductive failure amongst marine invertebrates (Dog-whelks, *Nucella lapillus*) is due to "imposex," the development of male characters, notably a penis, on females, caused by tributyltin (a tin-containing organic compound used in antifouling paints (Gibbs and Bryan, 1986; Gibbs et al., 1987).

Environmental factors affecting toxicity

Toxicity of a metal can vary over several orders of magnitude (Figs. 18.5 and 18.6) depending upon a number of factors, most notably pH and water hardness. These can affect the speciation of the metal in solution, the rate that it is accumulated by an organism and the organism's response. In particular, pH has a pronounced effect on speciation of some metals (e.g., Al, Cu, Mn and Pb; Campbell and Stokes, 1985), with the proportion present as free ions (generally regarded as the most toxic form) increasing as pH falls. For many other metals, however, pH-induced changes in toxicity are more likely to be due to the organisms response to the metals. Metal accumulation is much reduced at low pH (Satake et al., 1984; Harrison et al., 1986). However, the amelioration caused by less metal being accumulated at low pH values has to be considered alongside the increased physiological stress caused by low pH. This is well illustrated by a study on *Salmo gairdneri* (rainbow trout) in which Cu uptake at pH 5.0 was half that at pH 7.0 while toxicity increased between four and ten times (Laurén and McDonald, 1986).

These effects are particularly pronounced in "soft" water with low buffering capacity. Water hardness is defined as the sum of concentrations of Ca and Mg salts; increased concentrations of these salts have been shown to decrease metal uptake by aquatic bryophytes both in the field (Wehr and Whitton, 1983) and the laboratory (Wehr et al., 1987). Many studies have demonstrated reduced toxicity in hard water (Winner and Gauss, 1986; Mance, 1987) although the scale of the effect varies depending upon the metal in question. A likely explanation for these effects is increased competition for metal binding sites in the cell (Stokes, 1983).

Conversely, field (Harding and Whitton, 1976; Say et al., 1977) and laboratory (Harding and Whitton, 1977; Say and Whitton, 1977) studies have shown that algal populations from hard water are generally less tolerant to a particular Zn concentration than populations from soft water sites. This is presumably because the natural "protection" offered by Ca and Mg (which competes with the metals for binding sites and so reduces uptake) reduces the need for genetic tolerance mechanisms.

An external factor that might reduce the concentration of free metal ions is the presence of natural chelating agents such as humic acids and polypeptides, which have been shown to markedly increase LC₅₀ concentrations of metals to invertebrates (Winner, 1984; Winner and Gauss, 1986). EDTA is often used in the laboratory as an artificial chelating agent and it appears to mask the toxic effects of metals up to the point where the concentration of metal exceeds the binding capacity of the EDTA (Stokes, 1983; Schreinemakers and Dorhout, 1985). Chelating agents occur naturally, for example from the breakdown of plant products, but many algae also appear to produce their own chelating agents in response to elevated metal concentrations (see above; Jardim and Pearson, 1984; Starodub et al., 1987)

Phosphorus is an important factor determining toxicity of metals to algae and other plants. As the major nutrient limiting growth in freshwaters, P can have a marked effect on algae quite separate to any alleviation of metal toxicity (Say and Whitton, 1977): for example, phosphate-starved populations of '*Anacystis nidulans*' (\equiv *Synechococcus* sp.) have been shown to be more sensitive to Zn than phosphate-rich populations (Shehata and Whitton, 1982). However, interpretation of these results is difficult as high concentrations of phosphates (as found in recipes for many growth media) tend to form insoluble complexes with metals (Stumm and Morgan, 1981), rendering the metal unavailable to organisms, while other studies have shown metals to inhibit P uptake (Peterson and Healey, 1985) and/or metabolism (Kuwabara, 1985).

Synergism, additivity, and antagonism

Where two or more metals are found together, the combined toxic effect may: (1) equal the sum of the toxicities of the constituents ("additive" behavior); (2) exceed the sum of the toxicities of the individual metals ("synergistic" behavior); or (3) be less than the sum of the individual toxicities ("antagonistic" behavior). In practice, results are strongly affected by experimental protocols (Wong and Beaver, 1981; Kelly, 1988): for example, mixtures of Zn and Cd have behaved synergistically to algae (Say and Whitton, 1977) and higher plants (Hutchinson and Czyska, 1975), additively to some higher plants (Nasu et al., 1984) and invertebrates (Thorp and Lake, 1973), and antagonistically to some algae (Shehata and Whitton, 1982) and invertebrates (Thorp and Lake, 1973). By contrast, Cu and Zn appear to always act additively (algae: Petersen, 1982; invertebrates: McMurtry, 1984; fish: Thompson et al., 1980).

Metallothioneins: a unifying hypothesis for metal toxicity?

Metallothioneins are a group of proteins with molecular weight between 6000–7000 Daltons and 60–62 amino acids,

including about 20 cysteine residues (Robinson et al., 1993; George and Langston, 1994). Their original function remains the subject of debate, although it seems likely that they may bind surplus quantities of potentially toxic metals such as Cu and Zn which are essential for life processes, in order to maintain a small "store" (George and Langston, 1994). Under normal (i.e., unpolluted) conditions, metal accumulation works to an organism's advantage, permitting trace metals that might otherwise limit growth to be scavenged from the environment. Once inside a cell, however, they need to be stored in a non-toxic state until required for growth. However, where concentrations of Cu and Zn—or chemically similar metals such as Cd and Hg—are elevated, the capacity of the metallothioneins is exceeded and life processes will be inhibited. Organisms can, to some extent overcome this by increasing the amount of metallothionein in a tissue, and correlations have been demonstrated in fish between metallothionein concentrations and hepatic (George and Langston, 1994) and ambient (Roch and McCarter, 1984) metal concentrations.

Metallothionein-like compounds have been found in virtually all groups of organisms, with particularly strong homology (i.e., similar protein and DNA sequences) amongst the vertebrates (George and Langston, 1994) for which a vast literature exists. However, studies on fast-growing lower organisms such as fungi and algae which are easily manipulated in culture have provided several insights into the evolutionary and ecological significance of these compounds. It has been shown, for example, that the metallothionein gene can be deleted from fungi and cyanobacteria using molecular biological techniques, with little effect on growth or reproduction but with reduced tolerance to metals (Hamer, 1986; Turner et al., 1993). In other words, where metal concentrations are low and constant, then organisms do not need metallothioneins for maintaining a constant internal environment (homeostasis). When metal concentrations increase, however, then "normal" cells are able to increase the quantity of metallothionein by increased gene transcription (Hamer, 1986).

Where lines of cyanobacteria tolerant to Cd have been induced by sub-culturing into successively higher concentrations of metals, an increase in the gene copy number has been observed (Gupta et al., 1992), permitting more Cd to be bound within the cell. This was accompanied by the deletion of the repressor which normally controlled metallothionein synthesis (Gupta et al., 1993), which meant that the gene was permanently "switched-on" in Cd-tolerant cells.

Metallothioneins—and metallothionein-like compounds—are clearly widespread and may partly explain why organisms can accumulate relatively high concentrations of metals. To date, however, there have been few studies synthesizing these molecular studies with ecological and ecotoxicological observations. There appear to be, for example, no studies in which the effect on metallothioneins of environmental factors such as pH and hardness are evaluated. What follows is, therefore, speculative.

Many of the environmental factors which protect against toxic metals do so outside the cell, either by affecting the chemical speciation of the metal or by competing with the metal either for binding sites on the cell wall (Kelly, 1988) or for uptake sites on the cell membrane (see above). It might be postulated that they are acting in series with the metallothioneins, insofar as metals that are not trapped either outside the cell or (in the case of plants) in the cell wall, are absorbed and so will provoke metallothionein synthesis. Selective pressures will therefore work on all stages contributing to metal tolerance, and a study demonstrating metal

exclusion as a tolerance mechanism (e.g., Foster, 1977) does not necessarily invalidate hypotheses concerning metallothioneins. Indeed, in groups such as the aquatic bryophytes with thick cell walls rich in cation-exchange sites (Wehr et al., 1987), only 2% or less of metal was estimated to be bound to metallothionein-like compounds (Jackson et al., 1991).

CONCLUDING COMMENTS

It has been my intention in this review to identify some of the common strands from an enormous literature covering both a large number of metals and a huge variety of organisms. It should be apparent from what I have written that several factors are common, relating to broad chemical similarities between metals and common physiological mechanisms at the cellular level. These two factors form a contrapuntal theme around which infinite variations can be woven.

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