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Microorganisms and Heavy Metal Toxicity

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Abstract. The environmental and microbiological factors that can influence heavy metal toxicity are discussed with a view to understanding the mechanisms of microbial metal tolerance. It is apparent that metal toxicity can be heavily influenced by environmental conditions. Binding of metals to organic materials, precipitation, complexation, and ionic interactions are all important phenomena that must be considered carefully in laboratory and field studies. It is also obvious that microbes possess a range of tolerance mechanisms, most featuring some kind of detoxification. Many of these detoxification mechanisms occur widely in the microbial world and are not only specific to microbes growing in metal-contaminated environments.

Introduction

The heavy metals constitute a group of about 40 elements with a density greater than five (80). A feature of heavy metal physiology is that even though many of them are essential for growth, they are also reported to have comprehensively toxic effects on cells, mainly as a result of their ability to denature protein molecules. There are, however, many reports in the literature of microbial resistance to heavy metals. The phenomenon of microbial resistance is of some fundamental importance and is particularly relevant to microbial ecology, especially in connection with the roles of microbes in polluted ecosystems and in the reclamation of metal-contaminated natural habitats. It is also important to understand the mechanisms of microbial tolerance because of the extensive use of some metals and metal compounds as fungicides and disinfectants. It is the purpose of this review to examine the nature of the interactions between microbes and heavy metals and to attempt to clarify the processes, both environmental and microbial, underlying resistance or tolerance.

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Environmental Influence on Toxicity

Binding to Environmental Constituents

One of the most important factors that determines the biological availability of a metal in a system is its binding to other environmental constituents. If a metal is wholly or partially removed by binding, a decrease or complete disappearance of toxic effects may result.

In the soil, metals can be bound strongly by organic materials such as humic and fulvic acids and proteins. Humic acids are especially important and it has been stated that practically every aspect of the chemistry of heavy metals in soils, sediments, and natural waters is related in some way to the formation of complexes with humic substances (13,23,100). In some cases metal availability is limited by binding to humic acids to such an extent that deficiency symptoms may result in plants growing in such soils (30). The bound metal is often difficult to remove, and even in very sandy soils extraction can require vigorous procedures (46).

Clay particles can also bind metal cations, and some metals such as zinc may enter the crystal lattice and become unavailable to organisms (46,118). Clay particles can reduce the toxicity of certain metals toward microorganisms. Experiments with cadmium have shown that the clay minerals, montmorillonite and kaolinite, protected certain bacteria, actinomycetes, and filamentous fungi from the inhibitory effects of cadmium. This protective ability of the clays was correlated with their cation-exchange capacity (CEC) as it appeared that the greater the CEC, the greater the amount of cadmium absorbed (8,9).

In aquatic habitats, metals such as zinc and copper can be bound and removed from the water by organic sediments, which effectively reduces the total metal ion concentration in solution. It has also been reported that certain oxidized sediments can bind up to 96% of added zinc (11,114). Certain waters, especially those in moorland areas, contain considerable amounts of humic substances and, as in the soil environment, a variety of metals can be bound including zinc, cobalt, and mercury (13,83). Because of such binding in aquatic systems, it has been shown that toxic effects of certain metals on microbes can be decreased (70).

In certain polluted aquatic habitats, metals such as mercury can be trapped and bound by petroleum, and since many oil-degrading microbes are active at the oil-water interface, such removal is of obvious ecological significance in that it may enable the growth of metal-sensitive organisms. In one study of an oil-polluted marine habitat, it was found that the concentration of mercury in the oil was 4000 times higher than in the sediment and 300,000 times higher than in the water samples. Many of the oil-degrading organisms isolated were found to be mercury resistant, but the extent to which the mercury removal influenced resistant behavior was not determined (112).

Compounds which can chelate metals, for example, citrate, cysteine, glutamate, and EDTA, can also have a significant effect on microbial responses when included in growth media. Toxic effects of copper on *Aerobacter aerogenes* were prevented by the addition of yeast extract and cysteine, and this was attributed to the ability of these compounds to bind copper (66). Similarly, toxic

effects of copper ascorbate to *Serratia marcescens* were relieved by the addition of copper-chelating agents (119). Citrate and EDTA can reduce toxicity of some metals to *A. aerogenes*. In the presence of citrate this organism could grow in 200 ppm cadmium, but if glucose was substituted for the citrate an "infinite lag" resulted (82). A study of mercury toxicity using the protozoan *Tetrahymena pyriformis* revealed that toxic levels in a complex medium containing proteose peptone and liver digest were about 40 times higher than those observed in a simpler medium (44). Copper toxicity to *Anabaena cylindrica* has also been shown to be reduced by the addition of EDTA (35).

In media without complexing agents, toxicity may be pronounced. This is the case with *Chlorella pyrenoidosa* where a copper concentration as low as 5 $\mu g\ l^{-1}$ was toxic (99). These authors made the interesting suggestion that copper is not ordinarily present as the ionic form in natural waters but is usually complexed with organic materials such as polypeptides.

In brewery systems it has been commonly found that metals do not have the same effect on fermentation in simple and complex media (36,113). In general, fermentation is relatively unaffected by metal additions when tested in complex media. For example, in malt wort and molasses, a brewing yeast was unaffected by 30 to 40 ppm of copper, but the same yeast, when grown in a simpler sugar and mineral salts medium, was completely inhibited by 1 to 2 ppm copper (113).

In activated sludge, metals can be adsorbed by organic matter and a plant may be able to withstand quite high additions of metals without serious loss of activity. In one study, for example, it was found that protozoa were unaffected by copper concentrations up to 5 ppm and the reduction in overall efficiency was only 4% even at concentrations of 25 ppm (69).

It should be mentioned that although binding to environmental constituents usually reduces toxicity, in some cases toxic action still results even when there are no free metallic ions. This was found to occur in certain complex media with mercury. Although there were no free mercury ions until the total concentration was 160 ppm, a total concentration of 10 ppm was found to inhibit the growth of many aquatic bacteria (74,84). It was suggested that either the ions exerted their toxicity and entered the cell as organic complexes or bacterial cells competed successfully with the growth media for the bound ions (84).

Of course, in some cases metal complexes are more toxic than the free metal. This was shown for *Staphylococcus aureus* using 8-OH-quinoline (10^{-5} M) and ferrous ions (10^{-3} M). When these substances were applied separately, no toxic action resulted but a mixture completely inhibited growth (2). Although certain metal complexes are more toxic than the free metal, they are often volatile and may disappear from an environment. This is the case with methylated derivatives of mercury.

pH

pH can have a considerable effect on the availability and thus the toxicity of heavy metals in a given environment. In general, at an acid pH metals exist as free ionic cations, but at an alkaline pH the ionic cations precipitate as insoluble hydroxides or oxides. Most heavy metal hydroxides are insoluble. Copper, at

about 1 ppm, disappears from solution at any Eh when the pH is greater than 6, and at an Eh below +200 mV when the pH is less than 6. Zinc precipitates as zinc hydroxide, $Zn(OH)_2$, above pH 5, and above pH 8.5 it forms zincate ion which can be precipitated by calcium ions (27). The pH at which precipitation occurs varies among different metals and among oxidation states of the same element. Some metals, for example, copper, have more than one valence state and the oxidized state is favored by high pH. The hydroxides of these oxidized states are less soluble than those of reduced states and precipitate at low pH values (19). Thus low pH generally increases the availability of metal ions, whereas high pH decreases availability. This has been illustrated in soils, where in very acid conditions toxicity due to an abundance of iron, manganese, copper, and zinc can be removed by adding lime which raises the pH (19,46). The influence of pH on availability is also illustrated by a study of the toxicity of copper complexes to *Candida utilis*. Complexes with amino acids were less toxic at pH values of 7 than at lower pH values. It was suggested that at the lower pH, the stability of the complex was lessened, releasing free copper ions (7).

One aspect of pH and metal toxicity that should be mentioned is the occurrence of metal-polluted mine streams which are often very acidic (20,53,54,85). The low pH can arise from run-in from acid soils and also from the microbiological oxidation of sulfide-containing minerals by thiobacilli, for example, releasing sulfuric acid. The low pH can then release other metals such as lead, manganese, iron, and zinc into solution (114). The biology of such acid mine streams has received some study and it is evident that in spite of metal toxicity, there is still much microbiological life in the form of algae, bacteria, yeasts, protozoa, and fungi (14,26,39,114). Some bacteria are very tolerant indeed, such as the thiobacilli which can tolerate high concentrations of copper and zinc (107,114) and a *Pseudomonas* species which is highly tolerant of copper, manganese, and cobalt (68). It is not clear, however, whether the low pH is of any advantage to these organisms in reducing toxicity, or whether they are just extreme examples surviving by means of other tolerance mechanisms.

Ion Interactions

The biological activity of heavy metal ions can be markedly affected by the presence of other ions. Cations such as magnesium and calcium can often reduce heavy metal inhibition. Toxic effects of nickel, cobalt, cadmium, zinc, and manganese to *Escherichia coli* were decreased in media with a high magnesium content. The toxicity of nickel and cobalt to *A. aerogenes*, *Aspergillus niger*, and *C. utilis* was also diminished by magnesium. For all four organisms, using radioisotopes of nickel and cobalt, it was found that the high magnesium levels reduced the amounts of nickel and cobalt taken up by the cells (1). Inhibitory amounts of manganese, iron, cobalt, nickel, and copper to *Bacillus licheniformis* could likewise be antagonized by the addition of magnesium to the medium, although toxic concentrations of zinc and cadmium were less effectively reduced (42). Similarly, calcium and magnesium have been shown to reduce the toxicity of cadmium toward *A. niger* (61). It has also been found that the iron concentration in a medium had a detoxifying effect on copper to the alga *Chlorella*

pyrenoidosa. At the iron concentration used in algal growth media, copper may be adsorbed to the negatively charged micelles of ferric hydroxide (98).

Anions are able to reduce metal toxicity by precipitation. The hydroxyl ion has already been mentioned with regard to pH. Besides this, phosphate, thiosulfate, carbonate, and bicarbonate ions can form precipitates with heavy metals depending on their concentrations and the pH of the solution. The addition of such anions to growth media often reduces metal toxicity (89).

Sulfide, from hydrogen sulfide, can also prevent toxicity in many cases by precipitating the metal as an insoluble sulfide. Organisms that grow in or produce high sulfide concentrations, e.g., *Desulfovibrio desulfuricans*, have been shown to be unaffected by large additions of heavy metals (105). This mechanism of tolerance will be discussed later.

Sometimes toxicity of a metal is increased when other ions are present. In the case of the alga *Chlorella vulgaris*, an asymmetric respiratory response occurs when fluoride and copper ions are applied jointly; respiration is completely inhibited by a mixture, but individually the ions have little effect (45).

Mixtures of heavy metals often exert a more pronounced effect on microorganisms, e.g., a mixture of copper and silver ions on algae (117), but this can be accounted for by simple additive effects (114). Synergistic effects of metals on microbial growth and survival have, however, not received much attention.

Mechanisms of Microbial Resistance

Hydrogen Sulfide Production

Microbial hydrogen sulfide production often has significant effects on metal toxicity since most heavy metals form insoluble sulfides with H_2S. Consequently, H_2S-producing organisms often exhibit tolerance to heavy metals.

In yeasts, metal tolerance has often been linked with H_2S production, and the importance of such H_2S-producing yeasts in nature has often been documented (28). Copper- and mercury-tolerant strains of *Saccharomyces cerevisiae* produce more H_2S than do their nontolerant parent strains, the metals being precipitated as insoluble sulfides (58,72). Colonies of copper-tolerant strains appear black or dark brown in the presence of copper and contain much copper sulfide (5). Electron micrographs have shown that the copper sulfide was chiefly deposited in and around the cell wall (3,4,57). Similar precipitation, thought to be copper sulfide, has also been observed in the fungus *Poria vaillantii* (87).

Bacteria that are capable of H_2S production may exhibit tolerant behavior. The sulfate reducer *Desulfovibrio desulfuricans* produces H_2S, grows in high sulfide concentrations, and may be unaffected by the addition of high concentrations of heavy metals (105). Likewise, in anaerobic digesters sulfide reduces the toxicity of most heavy metals, the H_2S again resulting from bacterial reduction of sulfates (62).

It has been noted that in some cases sulfide-producing organisms can protect sensitive organisms from the toxic effects of metals. When *D. desulfuricans* was grown in mixed culture with a metal-sensitive strain of *Pseudomonas aeruginosa*, the latter organism could tolerate higher concentrations of mercurials than

it could in pure culture. Results indicated the H_2S produced by the sulfate reducer protected the pseudomonad (10). *S. aureus* was also found to exhibit a higher tolerance to mercurials when grown with *E. coli*. The protective effect of the *E. coli* was partly due to H_2S production and the extracellular production of glutathione (101).

Production of Organic Compounds

As previously mentioned, binding or chelation of a metal by organic substances present in the microbial environment can markedly affect metal toxicity. In some cases the microorganisms themselves are capable of producing such substances which may reduce toxicity. Citric acid, which can be produced by many yeasts and fungi, can readily chelate metal ions such as copper and may protect a fungus from copper poisoning (87). *A. niger* may be protected from the toxic effects of lead in this way (120). Oxalic acid production has been linked with copper tolerance of certain wood-rotting fungi. The fungi *Corrollus palustris, Serpula lachrymans,* and *Poria monticula* all produce copper oxalate crystals when grown on synthetic media containing copper sulfate (87). Oxalic acid is itself a toxic substance, but metals such as copper and iron, when complexed with it, remove its toxicity while losing their own. This has been observed with the oxalic acid-producing fungus *Endothia parasitica* (29).

Some mercury-resistant mutants of *Saccharomyces cerevisiae* were found to have a requirement for methionine. Evidence suggested that this compound, itself an efficient chelating agent, was used by the yeast to produce a "simple, diffusible substance" which acted as a detoxifying agent toward mercurials (93,94).

Intracellular organic substances can also determine metal tolerance. This was found to be the case with mercury-tolerant *A. niger* where a pool of intracellular sulfhydryl compounds complexed mercury and alleviated its toxic effects (6). Such sulfhydryl compounds have also been observed in copper-resistant yeasts (56,72).

Uptake and Accumulation

Microorganisms possess mechanisms by which metal cations can be taken up and accumulated from their environment. Although the amounts of metal cations needed for growth requirements are generally small, such uptake mechanisms can still operate at higher concentrations and can influence metal toxicity, toward both individual accumulating organisms and the microbial community. In general, if a metal is wholly or partly removed from a system by microbes, toxicity may be reduced. This kind of detoxification is similar to that which occurs if a metal is removed by environmental constituents.

There appear to be two main types of metal uptake by organisms. The first involves nonspecific binding of the metal to cell surfaces, slime layers, extracellular matrices, etc., whereas the second involves metabolism-dependent intracellular uptake.

The first type can be important since most heavy metals can be adsorbed onto the surface of microbial cells, both living and dead, and, in fact, the addition of dead bacterial cells to copper-inhibited laboratory cultures of bacteria is effective in reducing toxicity (39). With metals such as copper, cadmium, and zinc, complexation is possible with polygalacturonic acid, an important constituent of the outer layers of bacterial cells. The metal can be recovered from such complexes and the polymer regenerated (49). In yeasts, metabolism-independent surface binding is often to anionic groups of two species, polyphosphate and carboxyl, and such binding is rapid and reversible (79,88). Isolated cell walls of *S. cerevisiae* have been shown to bind their own weight of mercury to "high-affinity" sites (71). In the fungus *Neocosmospora vasinfecta,* surface binding of zinc to negatively charged groups on the hyphal surface was rapid, reversible, and temperature independent (81), as was the binding of zinc to *C. utilis* (31). Similar binding of cobalt by *Neurospora crassa* was also rapid and accounted for 30% to 40% of total metal uptake (110). Surface binding of metals may be especially important in slime-producing organisms or those organisms that grow in an extracellular matrix, the extracellular material acting as an "impermeable barrier." For example, zoogleoal bacteria, which are common in aquatic habitats, can survive and grow in the presence of high concentrations of heavy metal ions, the metals being adsorbed and precipitated within the extracellular matrix (37). Such organisms are effective in removing toxic ions from solution and are thus of great ecological significance in that they may allow more sensitive organisms to survive in a mixed community. However, they may present a hazard, especially if eaten by other organisms.

The second type of metal uptake, metabolism-dependent transport, has been studied in various algae and yeasts (16,31,32,38,75,76), bacteria (18,25,76), and fungi (81,110). A detailed account of the physiology of metal uptake will not be given here. It is, however, important to note that in most of the organisms studied, the amount of metal bound by surfaces is insignificant when compared to the amounts that can be taken up by energy-requiring processes (18,31,75,81). It should also be mentioned that most studies of metal uptake have been concerned with low micronutrient concentrations as opposed to higher concentrations where, in order to survive, an organism may have to express some mechanism of tolerance.

At higher concentrations, intracellular precipitation of the metal may occur after uptake. This itself can be a means of detoxification since the metal is compartmentalized and may be converted to another more innocuous form. For example, certain yeasts are capable of precipitating thallium within the mitochondria as thallium oxide. The oxide may subsequently be discharged from the mitochondria and excreted from the protoplast. This is termed oxidative detoxification (63,65). "Copper containing particles" have also been observed in *C. utilis* after growth in high copper concentrations (55). In another study, copper was used as a "stain" for electron microscopy since it was found that this metal attaches to the nucleoli and chromosomes of yeast (64). In the algae *Scenedesmus acutiformis* and *Scenedesmus acumitis,* precipitation of copper has been observed within vacuoles and nuclei when grown at higher cencentrations. At lower copper concentrations, electron-dense bodies containing copper were concentrated only in the nucleus (33). Precipitation of mercury in electron-dense

bodies has also been noted in the hyphae of the fungus *Chrysosporum pannorum* (115). Likewise, electron-dense bodies, presumed to contain zinc, have been observed in the fungus *Neocosmospora vasinfecta* after growth in a medium containing zinc (81). There is also evidence for the intracellular deposition of iron, as ferrous sulfide, within the sulfate-reducing bacteria *Desulfovibrio* and *Desulfotomaculum* (52). Crystals of a copper compound thought to be sulfide have also been observed in the mycelium of the fungus *Poria vaillantii* (87).

Decreased uptake or impermeability to a metal may be a means of resistance. Decreased uptake is the case with cadmium and *S. aureus* where a resistant strain takes up less cadmium than the sensitive parent strain (21,22,60,108). There is evidence that the genes for such cadmium resistance are located on extrachromosomal R-factors (plasmids) which are discussed later.

Impermeability is one explanation of tolerance for those fungi capable of growth in high copper concentrations. *Penicillium* and *Aspergillus* species have been found which can survive in saturated copper sulfate (12,97).

Metal Transformation

The biological transformation of certain heavy metals is an important process that can occur in many habitats and be carried out by a wide variety of microorganisms, chiefly bacteria and fungi. Metals cannot be broken down into other products but may, as a result of biological action, undergo changes in valence and/or conversion into organometallic compounds. Both processes can be considered to be detoxification mechanisms since volatilization and removal of the metal may result.

Transformations involving changes of valency have been chiefly studied with mercury. Several types of bacteria and yeast have been shown to effect the reduction of cationic mercury (Hg^{2+}) to the elemental state (Hg^0) (17,59,67,91). This usually results in the mercury being volatilized from the medium. Bacterial mercury resistance is closely linked with this volatilizing ability (109). The oxidation of elemental mercury to its cationic form can also be mediated by microbes. Bacteria shown to have this ability include *E. coli, Pseudomonas fluorescens, P. aeruginosa, Citrobacter* sp., *Bacillus subtilis,* and *B. megatherium* (47).

Transformation of certain metals into organometallic compounds by methylation is also an important detoxification mechanism. Metals that have been shown to undergo methylation include mercury (43,50,86), lead (34,116), cadmium, and tin (48). Methylation can be affected by the environmental factors mentioned in previous sections and also by the numbers and species of microbes present in a particular habitat. Methylation can be catalyzed by a wide variety of microorganisms: both aerobic and anaerobic bacteria (48,106,111,116), yeasts, and fungi (24,34,87,111). Although products of methylation may be more toxic than the free metal, they are often volatile and can be released into the atmosphere. This is the case with mercury and its methylated derivatives, methyl and dimethyl mercury (86).

Organometallic compounds can also undergo microbiological and chemical degradation which may result in the metal being reliberated, again usually in a

volatile form. This degradation can also be carried out by many kinds of microbes (51,74,91,96,106). Thus concentrations of metals and organometallic compounds in natural habitats may be reduced by microbial action.

Metal transformations have been shown to occur in a wide variety of habitats, e.g., lake and river sediments, soil, river water, and activated sludge, and in each case the microbial composition has been significantly different (90). As already mentioned, a wide variety of microbes can be involved in metal transformation, and the fact that a specific, transforming flora does not exist further emphasizes that the ability to transform is a widely occurring phenomenon and is the property of diverse and ubiquitous organisms from all kinds of habitats. Since the ability to transform and thus detoxify certain metals is a widely occurring phenomenon, it follows that metal resistance resulting from this ability will also be common.

Genetically Determined Metal Resistance

Bacterial resistance to some heavy metals can be controlled by genes on extrachromosomal resistance (R) factors or plasmids which can also control antibiotic resistance (77,92). Plasmids have chiefly been studied with regard to the transfer of antibiotic resistance, but it is now evident that drug and metal resistance are closely connected and often occur together in clinical isolates. For example, in a study using clinical isolates of *P. aeruginosa,* it was found that most of the metal-resistant isolates exhibited multiple resistance to metals and also to antibiotics, although a small number exhibited metal resistance only (73). It is also known that the penicillinase plasmids of *S. aureus* carry genes for resistance to certain metals as well as for penicillin resistance (60,78,95). Although many metals have been implicated in connection with plasmid-mediated resistance, only mercury and cadmium have been extensively studied, mainly because only with these metals do significant differences of resistance occur between sensitive and tolerant strains. This difference can be about 100-fold. With other metals, including lead, nickel, cobalt, and silver, the differences in resistance are small between sensitive and tolerant strains and make any physiological studies difficult (78,92).

Plasmid-mediated cadmium resistance has been much studied genetically, but surprisingly has only been shown to occur with *S. aureus* (92,95). As mentioned in a previous section, the cadmium resistance of *S. aureus* depends on decreased uptake of the metal by tolerant strains (21,22,60,108). There are some cases of cadmium transformation, but in these plasmids have not been implicated (48).

Plasmid-mediated mercury resistance appears to be more widespread than cadmium resistance and has been observed in enteric organisms, *S. aureus,* and *Pseudomonas* species (60,73,102,103). The mercury resistance depends on the ability of the organism to transform the mercury or organomercurial compound into an innocuous form (60). Mercury transformations have already been discussed, but it should be mentioned again that the ability to transform mercury has been observed in a wide diversity of bacteria from natural habitats (90), although plasmids have been implicated only in the bacteria mentioned above, i.e., enteric organisms, *S. aureus,* and certain *Pseudomonas* species. Most of

the studies on these have been with clinical isolates, where the possession of a metal-tolerating ability does not appear to have any survival importance (73,91,92).

This interesting phenomenon was also observed in some studies of antibiotic and heavy metal resistance patterns in staphylococci isolated from populations not known to be exposed to heavy metals or antibiotics. In such studies it was found that many strains exhibited multiple drug resistance and also multiple heavy metal resistance (40,41).

Nevertheless, plasmid-mediated metal resistance may be ecologically important since resistance can be rapidly transferred from resistant to sensitive bacteria. This has been shown for mercury-resistant *E. coli* where the genes for mercury resistance could be transferred to sensitive *E. coli* strains and also to *Aerobacter aerogenes* (59). Thus populations in natural habitats could adapt genetically to conditions of metal toxicity faster than by the processes of spontaneous mutation and natural selection (92).

Conclusions

Apart from the reports of the ability of some microbes to detoxify mercury and possibly some other metals by volatilization, the phenomenon of microbial resistance to heavy metal toxicity allows no simple explanation. This is undoubtedly due to the multiplicity of interactions that can occur between microbial cells, heavy metal ions, and other environmental constituents. In some instances, where components of the environment which may not be a direct result of microbial activity are responsible for detoxification, neither "resistance" nor "tolerance" are appropriate terms for describing the growth or persistence of microbes in the presence of high metal concentrations. Some laboratory experiments in which resistant microbial strains have been isolated and identified must be open to question, especially when the isolation or maintenance medium contains peptones, yeast extracts, or certain buffer solutions known to react chemically with heavy metals. It is not unlikely that under conditions such as these, resistance is only apparent and may be merely an indirect result of the interference with the availability of other nutrients to "metal-sensitive" cells.

The reduction of metal toxicity which may occur by the formation of complexes between metal ions and the surface or wall components of microbial cells is probably an example of gratuitous resistance in that, although it is a cell-mediated process, it does not depend on structures synthesized specifically to confer resistance on the cells. Extracellular precipitation of metal sulfides by hydrogen sulfide-producing organisms is probably another example of gratuitous resistance.

The reports of the intracellular accumulation of metals at high concentrations raises some further points of interest. It is likely that in these organisms, although the metal ions have actually passed across the cell boundaries, they are effectively localized and immobilized. Whether this particular mechanism is unique to eukaryotic cells in which intracellular compartmentation is much further developed than in prokaryotic cells is a subject that may be worthy of further investigation.

Two important points have emerged concerning the methodology used in investigations of microbial resistance to heavy metals. It is apparent that more care is needed in devising the cultural conditions under which microbial resistance is identified and assessed so that interference from complex organic molecules and even inorganic medium constituents is avoided or minimized. Furthermore, the protocols currently used to extract heavy metals from soils and surface waters have probably given rise to overestimates of their biologically effective concentrations. New procedures that will provide measurements of the biological, as opposed to the chemical, availability of heavy metals are urgent developments.

Finally, consideration of the microbe-metal-environment interactions is clearly of a more general interest to ecologists as it draws attention to the sorts of interactions that may occur in natural habitats between microbes and other nutrients.

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