Copper as a Biocidal Tool

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Abstract: Copper ions, either alone or in copper complexes, have been used to disinfect liquids, solids and human tissue for centuries. Today copper is used as a water purifier, algaecide, fungicide, nematocide, molluscicide as well as an anti-bacterial and anti-fouling agent. Copper also displays potent anti-viral activity. This article reviews (i) the biocidal properties of copper; (ii) the possible mechanisms by which copper is toxic to microorganisms; and (iii) the systems by which many microorganisms resist high concentrations of heavy metals, with an emphasis on copper.

1. COPPER AS BIOCIDE

Metal ions, either alone or in complexes, have been used to disinfect fluids, solids and tissues for centuries [1,2]. The ancient Greeks in the time of Hypocrates (400 BC) were the first to discover the sanitizing power of copper. They prescribed copper for pulmonary diseases and for purifying drinking water. Gangajal (holy water taken from the Ganges River) is stored in copper utensils in every Hindu household due to copper's anti-fouling and bacteriostatic properties. The early Phoenicians nailed copper strips to ships' hulls to inhibit fouling since cleaner vessels are faster and more maneuverable.

By the 18th century, copper had come into wide clinical use in the Western world in the treatment of mental disorders and afflictions of the lungs. Early American pioneers moving west across the continent put silver and copper coins in large wooden water casks to provide safe drinking water for their long journey. In the Second World War, Japanese soldiers put pieces of copper in their water bottles to help prevent dysentery. Copper sulphate is highly prized by some inhabitants of Africa and Asia for healing sores and skin diseases. NASA designed an ionization copper-silver water sterilizing system for its Apollo flights.

Copper is one of a relatively small group of metallic elements which are essential to human health. These elements, along with amino and fatty acids and vitamins, are required for normal metabolic processes. The adult body contains between 1.4 and 2.1 mg of copper per kg of body weight. It is estimated that a human eats and drinks about 1 mg of copper every day. After nutritional requirements are met, excess copper is released into bile and excreted in the feces [3].

Copper is considered safe to humans as demonstrated *inter alia* by the widespread and prolonged use of copper intrauterine devices (IUDs) [4,5]. In contrast to the low sensitivity of human tissue (skin or other) to copper [6], microorganisms are extremely susceptible to copper. Several mechanisms have been suggested to explain the toxicity of copper to microorganisms (see Section 2 below).

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1.1. Copper as a Bactericide

The bacteriostatic effect of copper was noted by Dr. Phyllis J. Kuhn [7], who was involved in the training of housekeeping and maintenance personnel at the Hamot Medical Center in Pennsylvania. To heighten their awareness of modes of infection, the students were given blood agar plates and instructions on their use. The students returned with bacterial cultures from such diverse sources as toilet bowl water (remarkably clean), salad from the employees' cafeteria (heavily colonized), and doorknobs. Brass (an alloy of 67% copper and 33% zinc) doorknob cultures showed sparse streptococcal and staphylococcal growth while stainless steel (about 88% iron and 12% chromium) doorknob cultures showed heavy growth of gram-positive organisms and an array of gram-negative organisms.

Based on this observation, Dr. Kuhn investigated bacterial growth on metals. Small strips of stainless steel, brass, aluminum, and copper were inoculated with broths of *Escherichia coli, Staphylococcus aureus, Streptococcus* group D, and *Pseudomonas* species. The broths contained a very heavy inoculum (~10^7 bacteria/ml). The strips were then air-dried for 24 hours at room temperature, inoculated onto blood agar plates, and incubated for 24 hours at 37°C. The results were striking. The copper and brass strips showed little or no growth, while the aluminum and stainless steel strips produced a heavy growth of all the different types of microbes.

The test was repeated at varying intervals of 15 minutes, 1, 5, 7, 20 and 24 hours. Brass disinfected itself in seven hours or less, depending on the inoculum size and the condition of the surface of the metal. Freshly scoured brass disinfected itself in one hour. Copper disinfected itself of some types of microbes within 15 minutes. Aluminum and stainless steel produced heavy growths of all isolates after eight days and growths of most isolates after three weeks.

In another experiment, stainless steel, aluminum, brass and copper strips were covered with seeded agar and incubated in culture for 24 hours. Replica plates from the stainless steel and aluminum strips allowed the growth of bacteria, while replica plates from the brass and copper strips did not. Scanning electron micrograph pictures of the surfaces of the metals showed that *E. coli* was completely disrupted on brass while remaining intact on stainless steel.

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More recently, the ability of various electroplated coatings (cobalt, zinc, copper, and cobalt-containing alloys of nickel, zinc, chromium, etc.) to inhibit the growth of pathogenic bacteria was examined [8]. Among the organisms used were gram-positive bacteria (*Enterococcus faecalis* and methicillin-resistant *S. aureus*) and gram-negative bacteria (*E. coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*). The amounts of H_2O_2 produced and metal ions dissolved from the surfaces of the various electroplated coatings were measured, and it was found that the inhibitory ability of the coatings correlated with the amount of H_2O_2 produced. The bacterial survival rates on the surfaces of the coatings were almost zero when H_2O_2 was produced in amounts greater than 10^{-6} mmol/cm^2. It was also noted that the concentration of metal ions dissolved from coatings were outside of the bacterial lethal range. In another study, it was shown that although H_2O_2 is toxic to bacteria in metal ion-depleted media, the lethal H_2O_2 dose level was sharply reduced if copper salts and reductants were added [9]. Similarly, it was found that the antibacterial potency of several compounds is significantly higher when they are complexed with copper [10,11]. Likewise, copper phosphate cement used as a restorative material for treatment of caries demonstrated the greatest *in vitro* and *in vivo* antibacterial activity, among several restorative materials tested [12,13]. Addition of activated copper significantly improved the antibacterial properties of calcium hydroxide used to kill bacteria in dentinal tubules [14].

The bacteriostatic effectiveness of copper used in paints to render surfaces self-disinfecting has also been demonstrated [15]. Nearly all of the copper paints tried were found to reduce the number of tested organisms (*S. aureus*, *E. coli*, *P. aeruginosa*, and *E. faecalis*) to negligible levels within 24 hours of exposure. This led to the use of copper in paints to reduce fouling and microbial biofilm formation in ships [16]. Fouling, the growth of barnacles, seaweed, tubeworms, and other organisms on boat bottoms, produces surface roughness that increases turbulent flow, acoustic noise, drag, and fuel consumption. An average increase of 10 μm in hull roughness can result in a 1% increase in fuel consumption!

Recently, the use of copper as a bacterial inhibitor in various stages of food processing has been demonstrated. It was shown that metallic copper surfaces inhibited the growth of two of the more prevalent bacterial pathogens that cause foodborne diseases, *Salmonella enterica* and *Campylobacter jejuni* [17]. Similarly, the addition of copper to drinking glasses has been shown to reduce biofilm formation of *Streptococcus sanguis*, reducing the risk of oral infections [18].

A platform technology has recently been developed, which binds copper to textile fibres from which woven and non-woven fabrics can be produced [19]. The ability to introduce copper into textile fabrics may have significant ramifications. Copper containing textiles may possibly be used to reduce healthcare-associated (nosocomial) infections in hospitals. Nosocomial infections rank fourth among causes of death in the United States, behind heart disease, cancer and stroke [20]. Nosocomial infections can be of bacterial, viral, fungal, or even parasitic origin. These infections are largely device-associated or surgically-related [21]. The main sources for contamination are the patient's skin flora, the flora on the hands of medical and nursing staff, and contaminated infusion fluids.

It has been demonstrated that sheets in direct contact with a patient's skin and a patient's bacterial flora are an important source of infection [22,23]. Moreover, sheets were significantly more contaminated by patients carrying infection than by non-infected patients [22]. Therefore, use of self-sterilizing fabrics in pajamas, sheets, pillow covers, and robes in a hospital setting may reduce the spread of microorganisms in hospital wards, which could result in a reduction of nosocomial infections.

An additional potential use of copper-impregnated fabrics is related to foot ulcerations, a common complication of type 1 and 2 diabetes, which afflicts approximately 130 million individuals around the world [24]. In many cases, these ulcerations can become highly infected due to cuts/bruises that do not heal, or heal very slowly. Infections that do not heal have been known to cause the tissue to die (gangrene). In severe cases, toes and legs may have to be amputated in order to save remaining healthy body parts. Use of socks containing copper-impregnated fibres by diabetics may significantly reduce the risk of foot infection by rendering the area aseptic. A preliminary clinical trial already indicates that such is the case (Dr. Richard Zatcoff, Upstate Podiatry, 110A Hospital Drive, Simpsonville, SC, USA, unpublished data).

1.2. Copper as a Water Purifier

Recognition of the bacteriostatic properties of copper has led to testing its capacity as a water purifier. Copper was found to be one of the most toxic metals to heterotrophic bacteria in aquatic environments. Albright and Wilson [25] found that sensitivity to heavy metals of microflora in water was (in order of decreasing sensitivity): Ag, Cu, Ni, Ba, Cr, Hg, Zn, Na, Cd. Sagripanti *et al*. [26] found that cupric chloride inactivated 9 of the 13 bacteria strains that they tested by more than 5 logs within 30 minutes. The other four strains were inactivated to a lesser extent.

In another study [27], water with a suspension of *E. coli* was introduced into 50 foot coils of different plumbing materials and changes in bacteria viability were periodically determined. While in different types of plumbing material, including glass, the level of bacteria remained the same or even increased, in the copper loop only 1% of the *E. coli* bacteria remained viable after five hours.

Similarly, it was found that water distribution systems made of copper have a greater potential for suppressing growth and for decreasing persistance of *Legionella pneumophila* cells in potable water than distribution systems constructed of plastic materials or galvanized steel [27]. The use of a continuous culture model system for the growth of *L. pneumophila* on copper and other plumbing materials was also examined. It was found that bacteria levels were reduced on copper surfaces compared with a glass control and other plumbing materials at the various temperatures tested and in the three different waters used [28].

A controlled evaluation was also made of the efficacy of copper-silver ionization in eradicating *L. pneumophila* from a hospital water supply. Copper-silver ionization units were

installed on the hot water recirculation line of a building positive for *Legionella*. Another building with the same water supply served as a control. *Legionella* species persisted within the system when copper and silver concentrations were < 0.3 and < 0.03 ppm, respectively. When copper and silver concentrations were > 0.4 and > 0.04 ppm, respectively, there was a significant decrease in *Legionella* colonization, but the percentage of water fixtures testing positive for organisms remained unchanged in the control building. When the ionization unit was inactivated, water fixtures continued to be free of *Legionella* for two additional months [29]. Similar results were obtained in other studies [30-33]. According to a report published in 1998, more than 30 hospitals in the USA are now using copper-silver ionization to control *Legionella* in their water distribution systems [34].

The efficacy of a 1:10 silver/copper combination for *in vitro* inactivation of *Hartmannella vermiformis* amoebas and the ciliated protozoan *Tetrahymena pyriformis* was also studied [35]. *Tetrahymena* and *Hartmannella* were inactivated by 2 log steps using a combination of 100 + 1000 μg/L Ag + Cu. The investigation clearly showed that levels within the limit of German drinking water regulations (10 + 100 μg/L Ag + Cu) could not inactivate these protozoa *in vitro*. It was further demonstrated that in the case of *Naegleria fowleria*, the organism responsible for primary amoebic meningoencephalitis, a combination of silver and copper ions (80 and 800 ug/L, respectively) was ineffective in inactivating the amoebae. However, addition of 1.0 mg/L free chlorine produced a synergistic effect, with superior inactivation relative to either chlorine or silver-copper used alone. A similar synergy was reported for *Staphylococcus sp.* and *P. aeruginosa* [36].

1.3. Copper as an Algicide, Fungicide, Molluscicide and Acaricide

Copper compounds are most extensively employed in agriculture. The first recorded use of copper in agriculture was in 1761, when it was discovered that seed grains soaked in a weak solution of copper sulphate inhibited seed-borne fungi. Within a few decades, the practice of treating seed grains with copper sulphate had become so general and effective that today infections of seeds with fungi are rare and are no longer of any economic importance.

The greatest breakthrough for copper salts as fungicides undoubtedly came in the 1880's with the development of a lime-copper formulation by the French scientist Millardet. He showed that spraying grapes and vines with a mixture of copper sulphate, lime and water renders them remarkably free of downy mildew. By 1885, his vintner's spray formulation was the fungicide of choice in the U.S. and was given the name of "Bordeaux mixture." Within a year or two of the discovery of Bordeaux mixture, Burgundy mixture, which derives its name from the district of France in which it was first used, appeared on the scene. Burgundy mixture is prepared from copper sulphate and sodium carbonate and is analogous to Bordeaux mixture.

Trials with Bordeaux and Burgundy mixtures against various fungus diseases of plants soon established that many plant diseases could be prevented with small amounts of copper applied at the right time and in the correct manner. From then onwards copper fungicides have become indispensable and many thousands of tons are used annually all over the world to prevent plant diseases.

The discovery that many algae are highly susceptible to copper sulphate led to its use by water engineers to prevent the growth of algae in potable water reservoirs. Copper sulphate is also employed to control green slime and similar algal scums in farm ponds, rice fields, irrigation and drainage canals, rivers, lakes and swimming pools. However, copper sulphate may be very toxic to fish. In addition, the sulphate ions tend to combine with hydrogen in an aqueous solution to form sulphuric acid, which is highly corrosive.

The environmental hazards resulting from copper build-up in sediments, and the need for high dosages, have led to the production of compounds that provide the copper in a chelated form [37-39]. Chelated copper is non-reactive with other chemical constituents in the water.

However, the use of copper sulphate for algae control is still very common [40], primarily because of its low cost and ease of application. Copper sulphate is also widely used to inhibit timber and fabric decay by rendering them unpalatable to insects and protecting them from fungus attack. Copper sulphate has been in use since 1838 for preserving timber and is currently the base for many proprietary wood preservatives. Copper is also used as the active ingredient in products that prevent roof moss formation [41].

Similarly to copper sulphate, copper-8-quinolinolate and some of its derivates have been shown to have fungicidal activity against *Aspergillus spp.* at concentrations above 0.4 μg/ml [42-45]. Since infection with this fungus is a major problem among immunocompromised patients, such as AIDS patients, this agent has been used to reduce environmental contamination of fungi in hospitals [46].

Use of copper by the wider population may also be beneficial for more benign conditions. About 15-20% of the population suffers from *tinea pedis* [47,48]. While there are many clinical presentations of *tinea pedis*, the most common is between the toes and on the soles, heels and sides of the foot. Although this fungal infection is not usually dangerous, it can cause discomfort, may be resistant to treatment, and may spread to other parts of the body or other people. Affected feet can also become secondarily infected by bacteria. Recently, it has been found that copper-impregnated socks may be useful in preventing and treating *tinea pedis* (Ref. [19] and Dr. Richard Zatcoff, unpublished results).

Another possible use of copper in fabrics is related to allergies and asthma. It is estimated that 15% of the general population suffer from one or more allergic disorders of which allergic rhinitis is the most common [49]. Allergic rhinitis affects an estimated 20 to 40 million people in the US alone. Similarly, nearly 15 million Americans have asthma, including almost 5 million children. Approximately 5,500 persons die each year from asthma [50]. Dust mites are considered to be an important source of allergen for perennial rhinitis and asthmatic attacks [51], and copper impregnated fabrics have been shown to destroy them [19]. Thus, elimination of house dust mites in mattresses, quilts,

carpets and pillows would be an important step in improving the quality of life of those suffering from dust-mite related allergies [19].

Copper sulphate has been found to be a potent molluscicide [52]. Control of snails may be an important strategy in fighting some human diseases, such as bilharziasis. This disease is caused by a trematode parasite, *Schistosoma mansoni*, which uses snails and humans as hosts. The International Copper Research Association screened 23 copper compounds, in addition to copper sulphate, for their effectiveness in killing snails in water of low- and high-alkalinity, with and without high levels of suspended solids. One compound, cupric chloride-bis-n-dodecylamine, was considerably superior to copper sulphate under all test conditions. A bivalent, organic complexed, copper nitrate was marginally superior to copper sulphate [52].

1.4. Copper Antiviral Activity

In 1964, Yamamoto and colleagues [53] reported the inactivation of bacteriophages by copper. In 1971, Jordan and Nassar [54] showed that copper inactivated infectious bronchitis virus. In 1974, Totsuka and Ohtaki [55] showed that the effect of copper sulphate on poliovirus RNA is proportional to its concentration, and that most amino acids except cysteine had a protective effect against copper as did Fe^{2+} and Al^{3+}. In 1974, Coleman and colleagues [56] reported that herpes simplex virus (HSV) type I was sensitive to silver. In 1992, Sagripanti and colleagues [57,58] found that cupric and ferric ions were by themselves able to inactivate five enveloped or nonenveloped, single- or double-stranded DNA or RNA viruses (phi X174, T7, phi 6, Junin, and HSV). The metals were even more effective than glutaraldehyde in inactivating the viruses. The metal virucidal effect was enhanced by the addition of peroxide, particularly for Cu^{2+}. In every case, the viruses were more resistant to iron-peroxidase than copper-peroxidase on a metal concentration basis. The inactivation of HSV by copper was enhanced by reducing agents.

The protective effects of metal chelating agents and catalase, the lack of effect of superoxide dismutase, and the partial protection conferred by free-radical scavengers suggest that the mechanism of copper-mediated HSV inactivation is similar to that reported for copper-mediated DNA damage [59].

Sagripanti and Lightfoote [60] reported that Human Immunodeficiency Virus Type 1 (HIV-1) was inactivated by either cupric or ferric ions when the virus was free in solution and also three hours after cell infection. Fifty percent inactivation of cell-free HIV-1 was achieved with Cu^{2+} at concentrations between 0.16 and 1.6 mM, or by Fe^{3+} at concentrations between 1.8 to 18 mM. Thus, the dose to inactivate 50% of infectious HIV-1 (IC_{50}) by Cu^{2+} or Fe^{3+} is higher than that reported for glutaraldehyde (0.1 mM), for sodium hypochlorite (1.3 mM), and for sodium hydroxide (11.5 mM). It is, however, significantly lower than that required for HIV-1 inactivation by ethanol (360 mM). Treatment of infected cells for 30 minutes at 20°C with 6 mM Cu^{2+} or Fe^{3+} completely inhibited the formation of syncytia and the synthesis of virus-specific p24 antigen in HIV-infected cells, while still preserving cell viability. The use of copper in free flow filters that deactivate HIV-1 and West Nile Virus has recently been reported. The copper filters reduced the infectious titers of both viruses by 5 to 6 logs [19].

Wong *et al.* [61] reported a 10^6-fold reduction in bacteriophage R17 infectivity due to RNA degradation in the presence of both ascorbate and Cu^{2+}. A study published in 2001 reported the inactivation of poliovirus and bacteriophage MS-2 in copper pipes containing tap water as a result of a synergistic effect between copper and free chlorine [62]. It was found that the log reduction/hour of the bacteriophage MS-2 in the presence of 400 μg/L of leached copper was 0.385, in 20 mg/L free chlorine 7.605 and with both copper and chlorine, 10.906. This suggests that an oxidizing agent such as chlorine or hydrogen peroxide is necessary to break open the virus protein coat and allow the copper to bind to and denature the nucleic acid.

The International Copper Association, Ltd. investigated the effects of copper on the survival of waterborne viruses [63]. It found that poliovirus was completely inactivated by copper sulphate (20 mg/L) in the presence of hydrogen peroxide (10 μM), confirming observations of a synergistic effect of copper ions in the presence of oxidizing agents. The effect was reduced by the presence of a protective agent, L-histidine. Other proposed protective agents, disodium hydrogen orthophosphate, bovine serum albumin and catalase, were found to be relatively ineffective. Similarly, the Association found that copper reduced coxsackie virus types B2 & B4, echovirus 4 and simian rotavirus SA11 infectivity by over 98%. It concluded that there does not appear to be any significant difference between the capacity of copper to inhibit the different types of virus tested. The polio, coxsackie and echo viruses may be expected to behave similarly as they are of a similar size and are all members of the enterovirus group. However, the rotaviruses are considerably larger (75 nm diameter as opposed to 28 nm) and belong to the reovirus group, a completely different family of viruses. This suggests that whatever the mechanism removing or inactivating the viruses, it is not dependent on subtle properties associated with the surface of the viruses.

In another study, the efficacy of copper and silver ions, generated electrolytically until reaching final concentrations of 700 μg copper and 70 μg of silver per liter, in combination with low levels of free chlorine, was evaluated for the disinfection of hepatitis A virus, human rotavirus, human adenovirus, and poliovirus in water [64]. There was little inactivation of hepatitis A virus and human rotavirus under all conditions. Poliovirus showed more than a 4 log titer reduction in the presence of copper and silver combined with 0.5 mg of free chlorine per liter or in the presence of 1 mg of free chlorine per liter alone. Human adenovirus remained active longer than poliovirus after undergoing the same treatment, although it remained active significantly less than hepatitis A virus and human rotavirus. The addition of 700 μg of copper and 70 μg of silver per liter did not enhance the inactivation rates after exposure to 0.5 or 0.2 mg of free chlorine per liter, although on some occasions it produced a level of inactivation similar to that induced by a higher dose of free chlorine alone. These data indicate that copper and silver ions alone in water systems may not

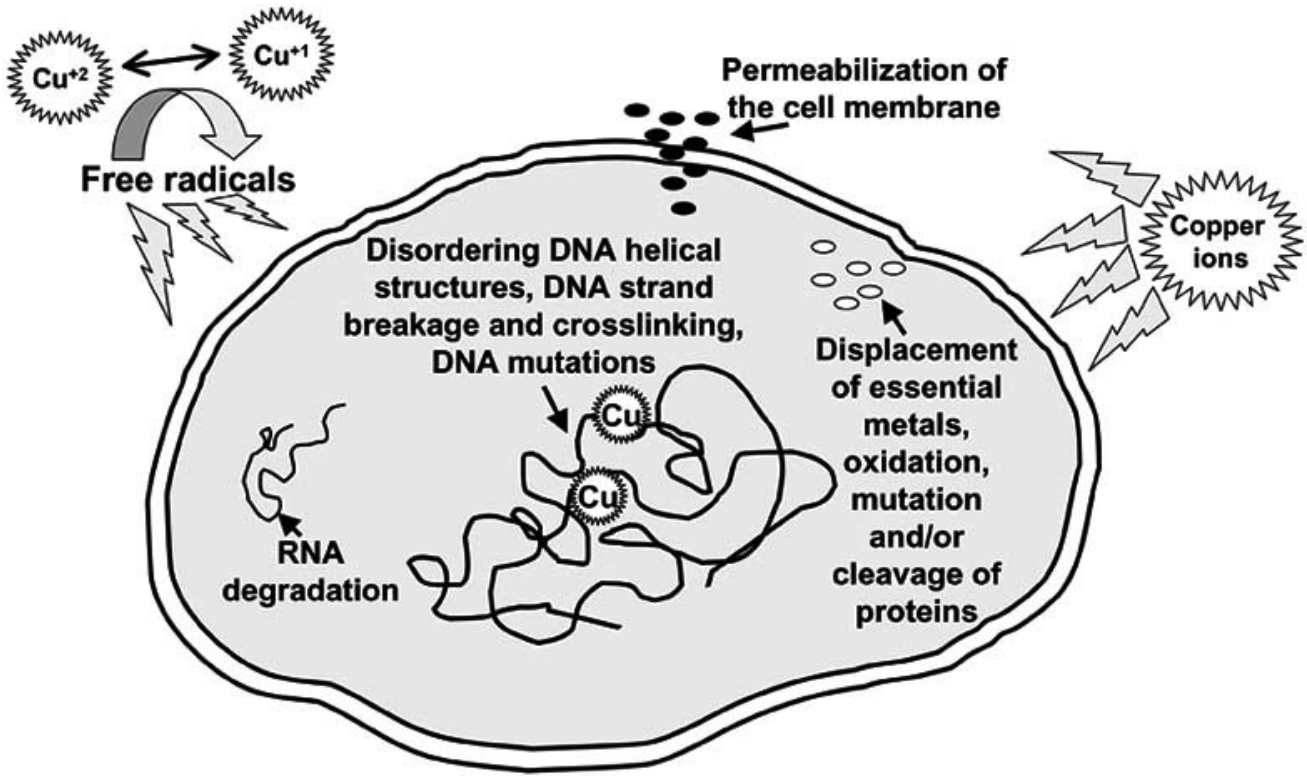

Fig. (1). Mechanisms of Toxicity of Copper to Microorganisms.

provide a reliable alternative to high levels of free chlorine for the disinfection of viral pathogens. It should be pointed out that the USA Environmental Protection Agency (EPA) has determined that drinking water should not contain more than 1.3 mg copper per liter of water (1.3 mg/L) [3].

2. MECHANISMS OF TOXICITY OF COPPER TO MICROORGANISMS

Metals at high concentrations are toxic to microorganisms. Toxicity occurs through several mechanisms (Fig. **1**), such as displacement of essential metals from their native binding sites or through ligand interactions. In general, nonessential metals bind with greater affinity to thiol-containing groups and oxygen sites than essential metals. Toxicity also results from changes in the conformational structure of nucleic acids and proteins and from interference with oxidative phosphorylation and osmotic balance. The redox properties that make some metals, such as copper, essential elements in biological systems, may also contribute to their inherent toxicity. For example, as shown in Fig. (**1**), redox cycling between Cu^{2+} and Cu^{1+} can catalyze the production of highly reactive hydroxyl radicals, which can subsequently damage lipids, proteins, DNA and other biomolecules.

2.1. Copper Mediated Cell Membrane Damage

Copper's initial site of action is thought to be at the plasma membrane [65-67]. It has been shown that exposure of fungi and yeast to elevated copper concentrations can lead to a rapid decline in membrane integrity. This generally manifests itself as leakage of mobile cellular solutes, such as potassium ions, and cell death. For example, exposure of intact *Saccharomyces cerevisiae* to Cu^{2+} (100 μM $CuCl_2$ in a buffer of low ionic strength) caused a loss of the permeability barrier of the plasma membrane within two minutes at 25ºC. The release of amino acids was partial, and the composition of the released amino acids was different from the composition remaining in the cells. Primarily glutamate was released, while arginine was retained in the cells. Cellular K^+ was released rapidly after the addition of $CuCl_2$, but 30% of the total K^+ was retained in the cells. These and other observations suggest that Cu^{2+} caused selective lesions in the permeability barrier of the plasma membrane but did not affect the permeability of the vacuolar membrane. These selective changes were not induced by the other divalent cations tested [66].

Similar effects reported in higher organisms have now been largely attributed to the redox-active nature of copper and the ability of copper to catalyze the generation of free radicals that promote membrane lipid peroxidation [67-70]. For example, Cu^{2+} uniquely catalyzed peroxidation of rat erythrocyte membrane lipid in the presence of 10 mM H_2O_2, while several other transition metal ions had no significant effect [70]. Thus, a copper-oxygen complex may be directly involved in the initiation of lipid peroxidation.

Extensive metal-induced disruption of membrane integrity inevitably leads to loss of cell viability. However, even relatively small alterations in the physical properties of biological membranes can elicit marked changes in the activities of many essential membrane-dependent functions,

including transport protein activity [71], phagocytosis [72], and ion permeability [71]. The physical properties of a membrane are largely determined by its lipid composition, one of whose important factors is the degree of fatty acid unsaturation. Microbial membrane fatty acid composition is highly variable and is influenced by both environmental and intrinsic factors. For example, the unsaturated fatty acid content of microorganisms generally increases at low temperatures [71,73]. In addition, some variation can be attributed to inherent differences in fatty acid composition between microbial groups [74]. The relationship between plasma membrane fatty acid composition and copper toxicity was studied in *S. cerevisiae.* It was found that copper-induced plasma membrane permeabilization and whole-cell toxicity increased markedly in cells enriched with polyunsaturated fatty acids [75].

In another study [9], the bactericidal potency of copper towards several bacteria (*Streptococcus lactis*, *E. coli* and *P. aeruginosa*) with different cell envelope structures was found to be similar. The authors suggested that the Cu^{2+} ion-mediated killing appears to be related to the bacterial plasma membrane. Their conclusion was based upon the observation that the loss of metabolic functions localized at the membranes paralleled cellular death, while oxidation of susceptible biomolecules within the cytosol required considerably more extensive oxidative degradation of the cells.

Many antibacterial and antifungal compounds are more active as copper salts [43,45,76-84]. It has been suggested that some compounds, such as diethyldithiocarbamic acid (DDC), which form chelates with copper and whose microbiocidal effectiveness is greatly enhanced by small amounts of copper, are cytocidal by virtue of concentrating in the lipid bilayer and, perhaps, by forming amphipathic complexes which disrupt membrane integrity [84].

2.2. Copper Interaction With Nucleic Acids

The study of radiation effects on diverse biological processes has led to the finding that microwave radiation at nonthermal levels produces single- and double-strand breaks in purified DNA. Interestingly, however, it was found that this microwave-induced damage to DNA depends on the presence of small amounts of copper. Moreover, it was found that cuprous, but not cupric, ions were able to mimic the effects produced by microwaves on DNA [85]. This finding has led to more detailed investigations into the *in vitro* effects of copper on DNA [88]. Today it is clear that Cu^{2+} has a specific affinity for DNA and can bind and disorder helical structures by crosslinking within and between strands [86,87]. Cu^{2+} reversibly denatures DNA in low ionic strength solutions competing with hydrogen bonding present within the DNA molecule [88]. Kinetic studies showed that the DNA double helix contains at least two kinds of binding sites for copper [89]. One site is present in every four nucleotides and has high affinity for copper. The other is an intercalating site for copper that is present in every base pair. This site is saturable and has a dissociation constant (K_d) for Cu^{2+} of 41 μM. In single-stranded DNA (ssDNA), such as that found in many DNA viruses, a copper binding site was found on average in every three nucleotides with lower affinity than in double stranded DNA (dsDNA) [89]. The binding of copper to DNA shows an unexpected high specificity when studied in the presence of other metallic ions. The relative efficacy of several different divalent cations to antagonize Cu^{2+} binding was: Ni = Cd = Mg >>> Zn = Hg > Ca > Pb >>> Mn, while Cr^{6+} enhanced Cu^{2+} binding to DNA [89].

Guanine-specific binding of Cu^{2+} in dsDNA was demonstrated following crystallization (1.2-A resolution) of DNA soaked with cupric chloride [90,91]. Covalent Cu^{2+} bonded to guanine occurred at the N-7 position. This preferential Cu^{2+} binding to guanine bases in dsDNA may explain the observed specificity of Cu^{2+}-induced oxidative DNA damage near guanine residues [92,93]. Hay and Morris [94] proposed that copper may stabilize the helix *via* a charge transfer complex formed when copper, acting as an electron acceptor, intercalates between two adjacent G-C pairs which act as electron donors. Indeed, copper has been shown to bind preferentially to G-C pairs [94].

DNA strand breakage is proportional to incubation time, temperature, and Cu^{2+} and H_2O_2 concentrations [92]. DNA strand breakage is inhibited by metal chelators, catalase, and by high levels of free radical scavengers implying that Cu^{2+}, Cu^{1+}, H_2O_2, and OH radicals are involved in the reaction. This, together with the specificity of copper binding to DNA (see above), has been interpreted to mean that nucleic acid degradation mediated by copper involves site-specific Fenton reactions [95-97]. Thus, subsequent to the specific binding of copper to nucleic acids, repeated cyclic redox reactions generate several OH radicals near the binding site causing multiple damage to the nucleic acids [67,98].

Cu^{2+} may also generate radicals and co-ordinate with other toxic molecules such as adriamycin, hydroquinone, and reduced mitomycin C [99-102]. For example, the addition of cupric acetate with adriamycin in the Ames salmonella mutagenicity test increases the mutagenicity of adriamycin by more than 700% [99]. This supports the contention that drug-metal ion-DNA associations might contribute to genotoxicity.

Glutathione, a chelating agent, was shown to inhibit free radical formation by copper ions in the presence of hydrogen peroxide, ascorbate and DNA [103]. The protective effect of glutathione was attributed to its ability to stabilize copper in the Cu^{1+} oxidation state, preventing redox cycling and generation of free radicals.

It has been shown that the DNA damage, which is the main cause of cell death in *E. coli* cultures treated with H_2O_2, occurs through iron-mediated Fenton reactions [104,105]. However, under conditions of low iron availability, copper ions take part in the genotoxicity of H_2O_2 in *E. coli* [106]. This phenomenon only occurs at high concentrations of H_2O_2 (20 mM), suggesting that copper ions only become available in the intracellular environment in the absence of iron and under severe oxidative stress [106].

DNA damage induced by many carcinogens is dependant on the presence of Cu^{2+} but not on the presence of other metals. The main modifications in the DNA occur at guanine and thymine residues, resulting mainly in the formation of 8-hydroxy-2'-deoxyguanosine and piperidine-labile sites at

thymine residues. There is a clear association between these modifications and DNA strand breakage [107].

It has been shown that in the cases of most carcinogens, copper-induced DNA damage occurs through the formation of H_2O_2 by the carcinogens [108-113], and that the reactive species generated by the reaction of H_2O_2 with Cu^{1+} participates in the DNA damage. In some cases the DNA damage is greatly enhanced by the reduction of the oxidized product through the addition of beta-nicotinamide adenine dinucleotide (NADH) [114-117]. These results suggest that an intermediate, derived from the reaction of Cu^{1+} with H_2O_2, participates in Cu^{2+}-dependent DNA damage, and that NADH enhances the DNA damage *via* a redox cycle.

A similar conclusion was reached by studying the mechanism of carcinogenesis caused by methylhydrazines [118]. Methylhydrazines in the presence of Cu^{2+} induce DNA cleavage at thymine residues only. The order of Cu^{2+}-mediated DNA damage was not correlated with the order of methyl free radical (CH_3) generation during Cu^{2+}-catalyzed auto-oxidation. It was therefore suggested that the Cu^{1+}-peroxide complex rather than the CH_3 plays a more important role in methylhydrazine plus Cu^{2+}-induced DNA damage. Mutations in DNA after exposure to copper ions were found to be clustered and predominantly single-base substitutions [119]. Electron spin resonance spin-trapping investigations demonstrated that hydrogen peroxide interacts with Cu^{1+} to generate the reactive species responsible for DNA damage, which is either the hydroxyl radical or a species of similar reactivity [120].

An important host defense mechanism for dealing with invading bacteria involves the production of reactive oxygen species, such as superoxide, hydrogen peroxide and hypochlorous acid, by phagocytic cells. Several antibacterial antibiotics have been shown to participate with transition metal ions in chemical reactions leading to the formation of reactive oxygen species. The production of reactive oxygen by redox cycling antibacterial antibiotics has led some investigators to suggest that a 'phagomimetic' contribution may also be made *in vivo*. Indeed, they showed that adding copper salts to four structurally different antibacterial antibiotics (beta-lactam, tetracycline, bacitracin and rifamycin) resulted in oxidative modification to bases in the bacterial DNA and enhanced antibacterial potency [121].

Epinephrine, norepinephrine or dopamine in the presence of non-lethal concentrations of Cu^{2+} kill washed or growing *E. coli* cells. This effect is enhanced by anoxia and by sublethal concentrations of H_2O_2. The rate of cell mortality was proportional to the rate of catecholamine oxidation. The copper epinephrine complex was found to bind to *E. coli* cells, to induce membrane damage and to deplete the cellular ATP pool. The cells were partially protected by superoxide dismutases (SOD) or catalase but not by OH radical scavengers. The addition of H_2O_2 to cells, which were sensitized by preincubation with the epinephrine-copper complex, caused rapid killing and DNA degradation [122].

2.3. Copper Mediated Protein Damage

Copper can alter proteins and inhibit their biological activities. For example, copper was the most potent metal inhibiting the protein tyrosine phosphatase VHR [123], a phosphatase that in conjunction with protein tyrosine kinases regulates cell growth and differentiation. Among the various metal ions (Fe^{3+}, Cu^{2+}, Zn^{2+}, and Cd^{2+}) examined for their inactivation effect on VHR, Cu^{2+} was found to be the most potent inactivator. The efficacy of Cu^{2+} as VHR inactivator was about 200-fold greater than that of H_2O_2. Cu^{2+} also inactivated other protein tyrosine phosphatases, including PTP1B and SHP-1. The Cu^{2+} -mediated inactivation was a consequence of the oxidation of a cysteine residue at the active site [123].

The reaction of a histidine-containing peptide (angiotensin I) with Cu^{2+}/ascorbate under physiological conditions has been studied [124]. In the presence of a catalytic amount of Cu^{2+}, ascorbate mediated the oxidative damage to the peptide *via* selective loss of the histidine residue. It was shown that the reaction of Cu^{2+}/ascorbate occurs specifically at the C-2 position of the imidazole ring of the histidine residue within a peptide. In addition, the capacity of copper to mediate free radical attack of proteins was investigated using proteins biosynthetically labeled with radioactive proline or histidine [125]. It was found that protein-bound histidine was substantially converted into aspartate, and while much of the proline was modified during radical attack, it was not converted into glutamate. Thus, histidine and proline are important sites of protein attack mediated by copper radical formation. Such protein modification may result in cleavage. For example, a facile cleavage of peptide bonds of apolipoprotein B (apoB) by radical reaction was found when human low-density lipoprotein (LDL) cholesterol was subjected to oxidative damage using Cu^{2+} [126]. When human serum was treated with Cu^{2+}, a similar cleavage pattern of apoB was also observed [126]. Similar selective attack at particular residues due to oxidative damage using Cu^{2+} was shown with bovine serum albumin (BSA), histones, cytochrome C, lysozyme and protamine [127].

It was suggested in a study of the biochemistry of copper [128] that the biocidal effects of copper are achieved mainly by the interaction of Cu^{2+} with SH- moieties present in the cell membrane and within cells. This interaction results in the formation of thiol compounds and Cu^{1+} ions. The following redox reaction

$$2Cu^{2+} + 6GSH \rightarrow 2Cu^{1}(GS)_2 + GSSG \text{ ; G= glutathione}$$

was suggested to occur when an excess of Cu^{2+} ions interact with living cells. This interaction leads to inactivation of 3 SH-groups per copper ion. This assumption was based on an equilibrium analysis of the Cu^{1+} - glutathione and Cu^{1+} - pencicillamine systems, in which it was found that reduced glutathione, which is present in all cells and cell membranes in concentrations from 1 to 6 mM, decreased markedly in the presence of copper.

2.4. Mechanisms of Copper Antiviral, Antifungal and Anti-Algae Activities

All of the above copper mediated toxic mechanisms, elucidated mainly in studies with bacteria, are relevant to viruses as well. For example, viral inactivation *via* RNA damage, through the Fenton mechanism, was proposed by Carubelli and colleagues [129]. They found that incubation of the RNA phage Q beta at 37˚C with a mixture of 100 mM

ribose and 10 μM $CuSO_4$ resulted in a complete loss of viable phage after 20 minutes. This cytotoxic effect required both ribose and cupric ions. There was a direct correlation between the decrease in phage survival and (a) the length of incubation, and (b) the concentrations of both ribose and $CuSO_4$. The addition of the strong chelator diethylenetriaminepentaacetic acid eliminated the cytotoxic effect. These results are consistent with an initial production of superoxide free radicals by transition metal catalyzed autoxidation of ribose and Amadori products (e.g. 1-amino-α-deoxyketose), followed by dismutation of the superoxide radicals to hydrogen peroxide and generation of lethal hydroxyl radicals by the Fenton reaction. RNA isolated from phage incubated with ribose and $CuSO_4$ retained its infectivity, suggesting that the cytotoxic effect may be mediated by a free radical attack on proteinaceous components of the phage through site-specific generation of hydroxyl radicals on protein-bound transition metal ions.

HIV-1 protease, an essential protein for the replication of the virus, was found to be inhibited by approximately stoichiometric concentrations of copper ions. Inactivation by Cu^{2+} is rapid and is not reversed by subsequent exposure to EDTA or dithiothreitol. The addition of copper to the protease at pH 5.5 induces aggregation of the protein, providing a possible basis for the inhibitory action of copper. Direct inhibition by Cu^{2+} required the presence of cysteine residue(s) in the protease. Oxygen is not required for inactivation [130,131].

In contrast to the different mechanisms of resistance to copper and other heavy metals found in bacteria, fungi and other microorganisms (see Section 3 below), viruses do not possess resistance or repair mechanisms, making them highly susceptible to high concentrations of copper ions.

The mechanism of copper antifungal and anti-algae activity has not been well studied. It has been suggested that the copper ions form electrostatic bonds with negatively charged regions on the microorganism's cell walls. These electrostatic bonds create stresses which lead to distorted cell walls and increased permeability, while also reducing the normal intake of life sustaining nutrients. Once inside an algae cell, copper may attack the sulphur groups in amino acids contained in the proteins used for photosynthesis. As a result, photosynthesis is blocked and cell lysis and death occurs [132].

3. MICROBIAL RESISTANCE TO COPPER

3.1. General

Many microorganisms demonstrate resistance to metals in water, soil and industrial waste. Metal resistance systems are present in nearly all bacterial types and may have developed shortly after prokaryotic life started. Several factors determine the extent of metal resistance in a microorganism. These include the type and number of mechanisms for metal uptake, the role each metal plays in normal metabolism, and the presence of genes located on plasmids, chromosomes, or transposons that control metal resistance [133-140]. Microorganisms may be resistant to one metal and not to another. For example, clinical staphylococcal isolates that demonstrate reduced susceptibility to lead and potassium are highly sensitive to silver and copper [141].

The mechanisms of tolerance include: exclusion by permeability barriers, intra- and extra-cellular sequestration by cell envelopes, active transport membrane efflux pumps, enzymatic detoxification, and reduction in the sensitivity of cellular targets to metal ions (Fig. **2**). Genes responsible for these processes may be encoded by the chromosomes or by plasmids. Since some toxic metals are also essential micronutrients (i.e. copper, cobalt, zinc, nickel), bacteria must precisely adjust their uptake and efflux systems to maintain adequate intracellular levels of these metals. In the case of metals with no biological function (i.e. cadmium, silver), transport systems must be oriented only to the expulsion of the toxic ions. In the last few years, several bacterial systems dedicated to toxic metal efflux have been analyzed at the molecular level resulting in a detailed understanding of the mechanisms. Among these are the membrane pathways that expel copper, cadmium, zinc, nickel, cobalt and silver cations. Two general mechanisms have been found: those involving P-type ATPases, and those using proton antiporter systems [135,140].

Natural resistance may take the form of mutations in cellular components that prevent interaction with metals or alterations in cell membrane composition. Microorganisms can possess one or a combination of several resistance mechanisms. There are six known resistance mechanisms to heavy metals in bacteria (see below). Each mechanism is discussed below, with emphasis on resistance to copper, and examples are provided of microorganisms that display the characteristics of each resistance mechanism.

3.2. Metal Exclusion by Permeability Barrier

Alterations in the cell wall, membrane, or envelope of a microorganism are examples of metal exclusion by a permeability barrier. This mechanism is an attempt by the organism to protect metal-sensitive cellular components. A prominent example is the exclusion of Cu^{2+} resulting from altered production by *E. coli* B of the membrane channel protein porin [142]. This is usually a result of a single mutation in the gene that regulates the permeability of the membrane to metal ions.

Bacteria that naturally form an extracellular polysaccharide coating demonstrate the ability to bioabsorb metal ions and prevent them from interacting with vital cellular components. For example, a layer of exopolysaccharide improves the survivability of *Klebsiella aerogenes* strains in Cd^{2+} solutions [143]. Nonspecific binding of metals by the outer membrane or envelope, such as in *Klebsiella aerogenes*, *Pseudomonas putida*, and *Arthrobacter viscosus,* however, offers limited metal protection due to the possibility of the saturation of binding sites [144,145].

Although not fully proven, it is believed that some forms of copper resistance are based on periplasmic binding [146,147]. Periplasmic binding of Cu^{2+} is thought to be found in *Pseudomonas* species where resistance is coded for in a plasmid by an operon of four genes: *copA*, *copB*, *copC*, and *copD* [148-150]. *CopA* and *copB* confer partial resistance, while the addition of *copC* and *copD* provides for

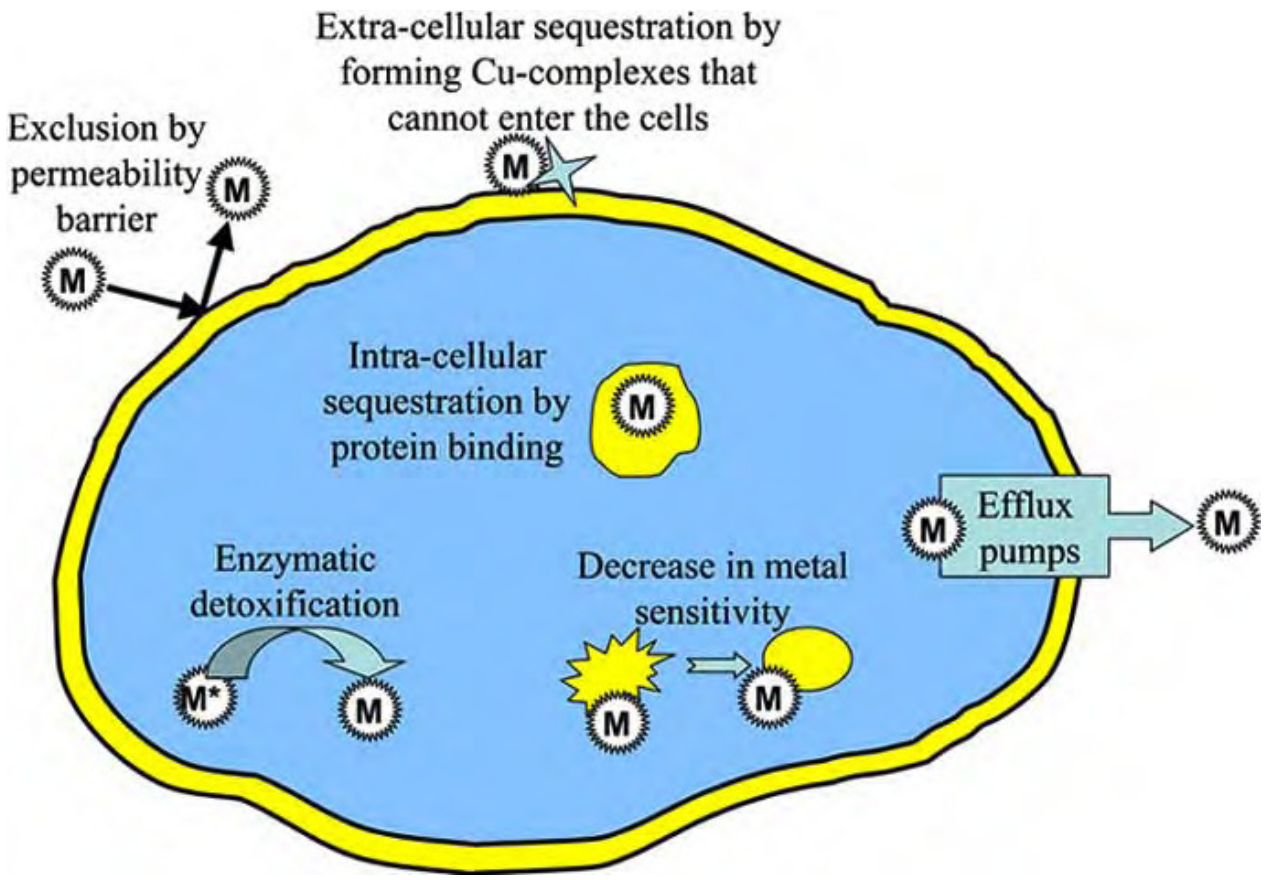

Fig. (2). Mechanisms of Microbial Resistance to Heavy Metals.

full Cu^{2+} resistance [146]. CopA and copC proteins are located between the inner and outer membrane, while copB is found in the outer membrane and copD in the inner membrane. CopC can bind Cu^{1+} and Cu^{2+} at two different sites, occupied either one at a time or simultaneously. The two sites are approximately 30 A apart and have little affinity for the ion in the other oxidation state. Oxidation of Cu^{1+}-CopC or reduction of Cu^{2+}-CopC causes migration of copper from one site to the other [149]. In addition, periplasmic proteins (e.g. PcoC) have been thought to bind copper ions and to increase the level of resistance to copper ions above that conferred by *cop* gene products [151-153].

3.3. Active Transport of the Metal Away from the Microorganism

Active transport or efflux systems represent the largest category of metal resistance systems. Microorganisms use active transport mechanisms to export toxic metals from their cytoplasm. These mechanisms can be chromosomal or plasmid-encoded. Non-essential metals normally enter the cell through normal nutrient transport systems but are rapidly exported. These efflux systems can be non-ATPase or ATPase-linked and highly specific for the cation or anion they export.

Active transport of the essential metal ion Cu^{2+} away from the bacteria is achieved through an ATPase efflux mechanism. The *cop* operon has been found in the gram-positive bacteria *Enterococcus hirae* and contains four genes: *copA*, *copB*, *copZ*, *copY*. The operon has nomenclature similar to that of the *cop* sequestration operon found in *Pseudomonas* discussed above. *CopA* is responsible for encoding a Cu^{2+} uptake ATPase and *copB* encodes a P-type efflux ATPase. The gene products of *copY* and *copZ* regulate the *cop* operon. CopY is believed to be a repressor protein that inactivates the operon in the absence of Cu^{2+}. When Cu^{2+} is present it may bind to copY, converting it to a DNA-binding repressor. Copper also binds to copZ at higher levels and together they form a copY-Cu complex that ceases repression. This allows *copA* and *copB* to be transcribed [154-157].

Recently, data has accumulated showing that *E. coli* encodes four proteins (CusC/F/B/A) that mediate resistance to copper and silver by cation efflux. All four proteins form a tetrapartite resistance system, which involves a novel periplasmic copper-binding protein CusF that directly transports Cu^{1+} from the periplasm across the outer membrane [158,159]. Data supporting a copper efflux system in *S. enterica serovar Typhimurium* has also been reported [160].

3.4. Intracellular Sequestration of Metals by Protein Binding

Intracellular sequestration is the accumulation of metals within the cytoplasm to prevent their exposure to essential cellular components. Metals commonly sequestered are Cu^{2+}, Cd^{2+}, and Zn^{2+}. Two examples exist for this form of metal resistance: metallothionein production in *Synechococcus* sp. and cysteine-rich proteins in *Pseudomonas* species

[142,157]. The metal resistance system in *Synechococcus* species consists of two genes; *smtA* and *smtB*. The gene *smtA* encodes a metallothionein that binds the metals. This gene is induced by high levels of Cd^{2+}, Zn^{2+}, and Cu^{2+} and is repressed by the gene product of *smtB*. Cysteine residues in SmtA metallothionein may act as a sink for excess toxic cations. Another organism that demonstrates intracellular Cd^{2+} sequestration is a strain of *Pseudomonas putida* that was isolated from sewage. This organism produces three low-molecular-weight cysteine-rich proteins, which may be related to metallothioneins.

Mycobacterium scrofulaceum also demonstrates intracellular accumulation of Cu^{2+} by sequestering it in the form of a black copper sulphate precipitate [161].

3.5. Extracellular Sequestration

Metal resistance based on extracellular sequestration has been found in several species of yeast and fungi and has been hypothesized for bacteria [162]. For example, *S. cerevisiae* secretes large amounts of glutathione. Glutathione binds Ni^{2+} with great affinity. The glutathione-metal complex cannot traverse the cell membrane, thus conferring the yeast resistance to Ni^{2+} [163]. Other organisms, such as *Citrobacter* species, form insoluble complexes of cadmium phosphate to confer cadmium resistance [164]. A similar mechanism exists in Cu^{2+}-resistant fungi, which secrete oxalate to form a metal-oxalate complex [140].

Interestingly, it has been shown that *P. aeruginosa* biofilms were anywhere from 2 to 600 times more resistant to heavy metals than free-swimming cells. The exterior of the biofilm was preferentially killed after exposure to elevated concentrations of copper, while the majority of cells that remained alive were in the substratum. A possible explanation for this phenomenon is that the extracellular polymeric substances that encase a biofilm may be responsible for protecting cells from heavy metal stress by binding the heavy metals, retarding their diffusion within the biofilm [165].

3.6. Enzymatic Detoxification of a Metal to a Less Toxic Form

There are several examples of enzymatic detoxification of metals to less toxic forms in microorganisms. Resistance to mercury may be achieved by enzymatic detoxification in both gram-positive and gram-negative bacteria. A set of genes encodes for the production of a periplasmic binding protein and membrane-associated transport proteins. The periplasmic binding protein collects Hg^{2+} from the surrounding environment and transport proteins take it to the cytoplasm where it is neutralized by redox chemistry [140].

Recently, a gram-negative bacterium, a pseudomonad very similar to *Pseudomonas synxantha* (CRB5), was isolated from a chromium-contaminated site. It was found to reduce toxic hexavalent chromium Cr^{6+} (chromate) to an insoluble Cr^{3+} precipitate under aerobic and anaerobic conditions. CRB5 tolerated up to 520 mg of Cr^{6+} per liter and reduced chromate in the presence of copper and arsenate. Under anaerobic conditions it also reduced Co^{3+} and U^{6+}, partially internalizing each metal. Metal precipitates were also found on the surface of the outer membrane. Chromate reduction by CRB5 is mediated by a soluble enzyme that was largely contained in the cytoplasm but also found outside of the cells. Membrane-associated Cr^{6+} reduction under anaerobiosis may account for anaerobic reduction of chromate under nongrowth conditions in the presence of an organic electron donor [166,167].

Metal ion-specific reducing enzyme systems function in the cytoplasm and cell surface layer of microorganisms [168]. These enzymes require NADH or NADPH as an electron donor and flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN), as an electron acceptor. Electron transport may be controlled by transplamsa membrane redox systems.

3.7. Decrease in Metal Sensitivity of Cellular Targets

Some microorganisms adapt to the presence of toxic metals by altering the sensitivity of essential cellular components. Protection is achieved by mutations that decrease sensitivity to a toxic metal but do not alter basic cellular function; alternatively, the mutations may increase production of a particular cellular component to keep ahead of metal inactivation. The microorganism may also protect itself by producing metal-resistant components or alternate pathways in an effort to bypass sensitive components.

DNA repair mechanisms may also provide limited protection to metal-induced damage of plasmid and genomic DNA. For example, upon exposure to Cd^{2+}, unadapted *E. coli* demonstrate considerable DNA damage; however, after subculture, the same organisms show resistance. The growth lag phase of the organism decreases the longer it is exposed to Cd^{2+}. It is postulated that the extended lag phase is initially due to a period of induction of DNA repair mechanisms.

Natural resistance can result from normal cellular functions that give the organism a base level of tolerance [142]. An example is naturally occurring glutathione, which may offer protection to metal ions like Cu^{1+}, Cu^{2+}, Ag^{1+}, Cd^{2+}, and Hg^{2+} [169]. Glutathione may offer protection from Cu^{2+} and Fe^{2+} by suppressing free radical formation [142].

There appear to be differences in the ability of gram-negative and gram-positive bacteria to tolerate certain metal ions. Gram-negative bacteria (*E. coli* and *Pseudomonas* species) are better able to carry on protein synthesis in the presence of Cd^{2+} than gram-positive bacteria. A species of *Pseudomonas* was shown to tolerate 5 to 30 times more Cd^{2+} in growth media before protein synthesis was reduced by 50% in comparison to gram-positive *S. aureus*, *Staphylococcus faecium*, and *Bacillus subtilis* [170]. Gram-positive organisms are able to bind 28 to 30 times more Cu^{2+} than *E. coli* [140].

Copper tolerance in fungi has also been ascribed to diverse mechanisms involving trapping of the metal by cell-wall components, altered uptake of copper, extracellular chelation or precipitation by secreted metabolites, and intracellular complexing by metallothioneins and phytochelatins. Only the metallothionein chelation mechanism has been studied in detail [65].

4. SUMMARY

In contrast to the low sensitivity of human tissue to copper [3-6], microorganisms are extremely susceptible to copper. Copper toxicity in microorganisms may occur through the displacement of essential metals from their native binding sites, from interference with oxidative phosphorylation and osmotic balance, and from alterations in the conformational structure of nucleic acids, membranes and proteins. In most microorganisms, but not in viruses, there is an integrated set of proteins that delivers copper to specific subcellular compartments and copper-containing proteins without releasing free copper ions. Although some organisms have mechanisms of resistance to excess copper, generally exposure of most microorganisms to high concentrations of this trace element results in damage to cellular components. Viruses lack DNA repair mechanisms, permeability barriers, intra- and extra-cellular sequestration of metals by cell envelopes, active metal transport membrane efflux pumps, and enzymatic metal detoxification mechanisms, such as those found in bacteria and cells. The reduced capabilities of viruses to resist copper may thus explain their high vulnerability to the metal.

Copper is used today as a biocide, mainly in agriculture. However, based on the above, further applications of copper in improving health are possible. One example may be the reduction of nosocomial infections in hospitals by the use of self-sterilizing copper-fabrics in pajamas, sheets, pillow covers, and robes. Similarly, the use of copper containing gloves with anti-bacterial and anti-viral properties may also aid in reducing nosocomial infections while providing increased protection to hospital personnel. As discussed in Sections 1.1 and 1.3, other possible uses of copper fabrics include the reduction of foot ulcerations, *tinea pedis* infections and dust-mite related allergies.

Another important potential application of copper-impregnated materials is related to the reduction of bacterial and viral transmission during transfusion of blood or blood-related products. The safety of whole blood and its components is a continuing global problem [171,172]. A growing number of viral, bacterial, and protozoa pathogens have been identified in blood products and new pathogens are regularly identified as present. The capacity of HIV-1 to be transported in whole blood by platelets and red blood cells has been demonstrated [173,174]. In areas of the world where screening tests are too expensive to be performed regularly, a cheap, rapid virus inactivation filter would be extremely helpful. Even in the US, hospitals can no longer afford to pay for expensive tests for each 'pathogen *du jour*' [175]. Accordingly, a filter that can inactivate a broad spectrum of viruses in blood products would be very valuable. However, it must first be established that these filters do not damage filtered plasma and other blood components and that they do not harm individuals infused with these blood products.

Yet another application of such generic antiviral filters is related to the reduction of HIV-1 transmission through breast-feeding, which accounts for one third to one half of all HIV mother-infant transmissions [176]. Breast milk may be passed through a copper fiber-containing filter reducing HIV infectivity. If there is little degradation to the milk's essential nutrients as a result of filtration, the filtered milk may be fed to infants thereby reducing the risk of HIV transmission. A similar application includes the neutralization of cytomegalovirus (CMV), which is transmitted through breast-milk. CMV causes severe maladies especially in premature or other high-risk infants.

In conclusion, there are many potential uses of copper in new applications that address medical concerns of the greatest importance. Implementation of even a few of these possible applications may have a major effect on our lives.

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